The Yale Journal of Biology and Medicine

The GEORGE HATHORN SMITH Number

Vol. 25, No. 5 April, 1953 New Haven, Conn.

THE YALE JOURNAL OF BIOLOGY AND MEDICINE

Founded in 1928

GEORGE H. SMITH

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Subscriptions. THE YALE JOURNAL OF BIOLOGY AND MEDICINE is published in September, November, December, February, April, and June from 333 Cedar Street, New Haven 11, Connecticut, U.S.A. The subscription price for one volume of six issues is $6.00 in the United States, $7.50 in other countries. For students registered in any school of Yale University, the subscription price is $4.00 per volume. Single copies are $1.50 each except for special issues.

Entered as second-class matter January 11, 1929, at the post office at New Haven, Connecticut, under the Act of March 3, 1879.

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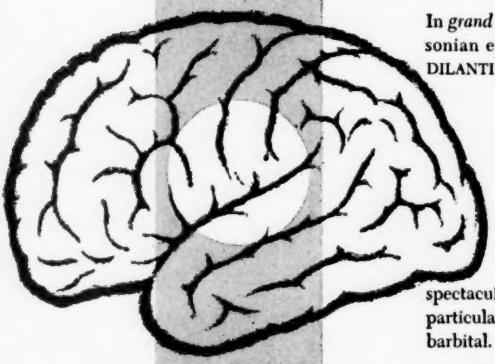

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(1) Krantz, J. C., and Carr, C. J.: The Pharmacologic Principles of Medical Practice, Baltimore, The Williams & Wilkins Company, 1949 (Reprinted 1950), p. 518. (2) *ibid*, p. 515. (3) Carter, S.: Epilepsy, in Conn, H. F.: Current Therapy 1952, Philadelphia, W. B. Saunders Company, 1952, p. 612. (4) Salter, W. T.: A Textbook of Pharmacology, Philadelphia, W. B. Saunders Company, 1952, p. 231.

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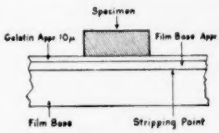

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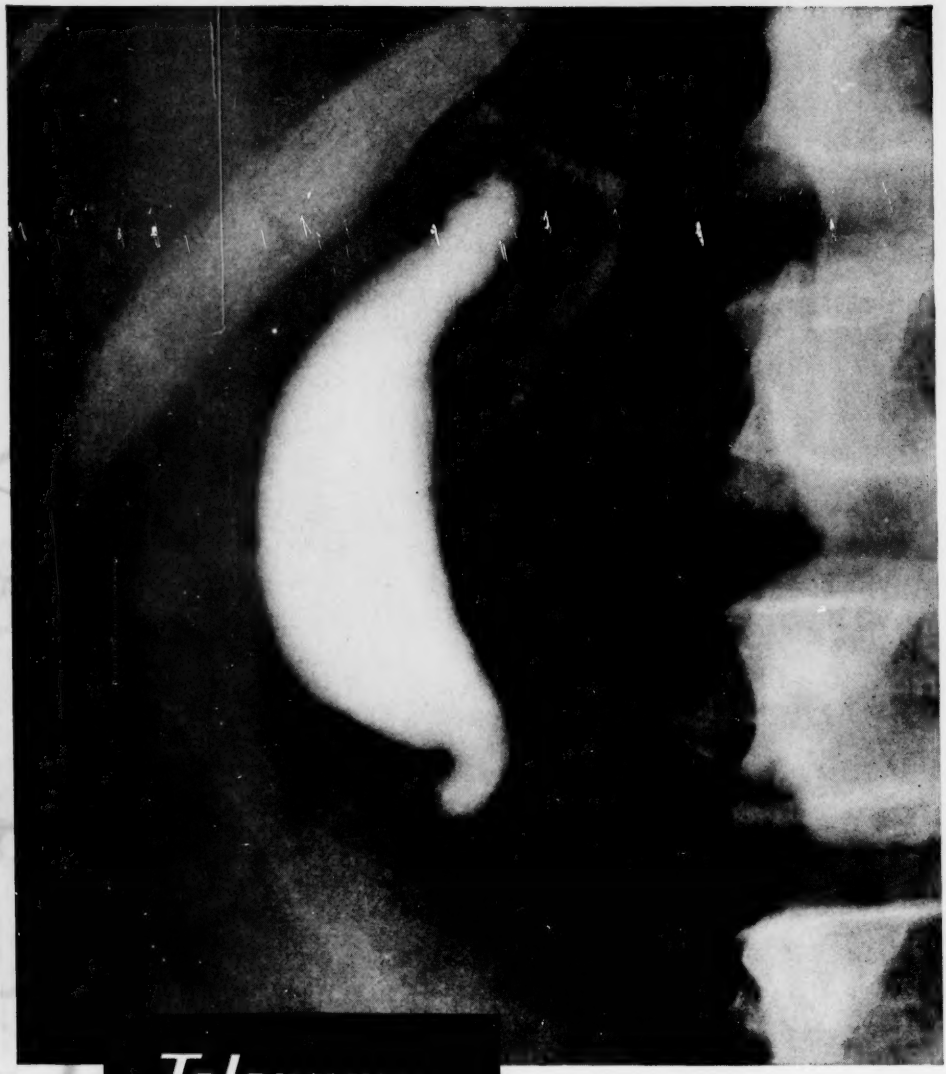
The Editors regret that the word "decrement" was omitted in Table 3 in the paper by Miller and Goodner on the Stability of Lyophilized BCG Vaccine in the February 1953 issue. The legend on page 269 should have read: Negative Log_{10} Decrement in Titer Immediately after Lyophilization, in the Presence of a Selected Series of Adjuvants.



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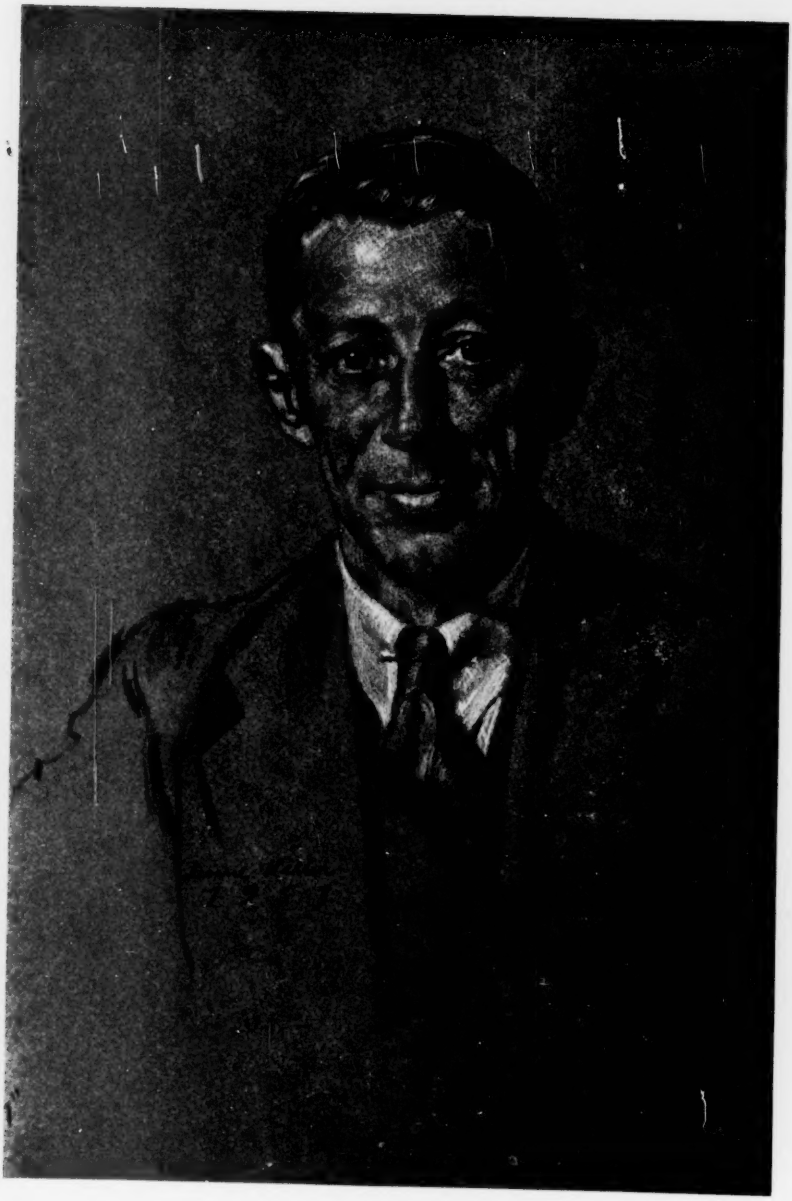

GEORGE HATHORN SMITH

(Reproduced by courtesy of the Yale University Art Gallery)

GEORGE HATHORN SMITH
1885-1952*

SAMUEL C. HARVEY**

It is altogether fitting that this issue of The Yale Journal of Biology and Medicine should be dedicated to the memory of its founder and editor-in-chief from 1928 to 1949. From him came "the inspiration, the continuity, the cohesiveness of purpose and effort which have brought the Journal an international reputation as a scientific publication . . ."; indeed, "the time and effort which Dr. Smith devoted to nurturing the Journal through difficult years have fashioned for him an enduring and worthy monument."[1]

Important as this contribution was to the Yale University School of Medicine, it would be sorely incorrect to ignore or minimize his other activities during those pregnant and at times tumultuous years which led to the renascence of the school. Previous to the advent of George Smith, whom Milton C. Winternitz—the newly appointed Professor of Pathology—brought with him in the fall of 1917, the then Dean and Professor of Medicine, George Blumer, had prepared the way for the future developments. He had engaged the University and the New Haven Hospital in contracts which opened the latter to the control of clinical material for teaching with the proviso that the heads of the Departments of Medicine, Surgery, and Obstetrics and Gynecology should be on a full-time basis, and had obtained funds to initiate this and to build the Anthony N. Brady Memorial Laboratory.[2] Smith's appointment was that of Assistant Professor of Bacteriology in the Department of Pathology and his laboratory was tucked in with those of Obstetrics and Gynecology, Medicine, and Public Health in the "Brady."

Smith, who was thirty-two years of age, came directly from the Research Laboratories of the H. K. Mulford Company where he had been for five years engaged in some eight projects, five of these having to do with "immunology." Parenthetically, the remuneration that induced him to accept this appointment at Yale was of an order that suggests a marked dislike for his previous employment or great longing for the so-called academic atmosphere. His preparation for teaching and research had been at Brown University from which he received a master's degree in 1910, and a Doctor of Philosophy in 1914. He had previously been graduated from

* From the Department of the History of Medicine, Yale University School of Medicine.

** William H. Carmalt Professor of Surgery Emeritus.

[1] Thomson, Elizabeth H. The twenty-fifth anniversary of The Yale Journal of Biology and Medicine. Some notes on its history. Yale J. Biol., 1952, *25*, 1.

[2] Blumer, George. Brief recollections of the Yale Medical School (1906-1920). Yale J. Biol., 1949, *21*, 374.

Bates College in 1909, having been prepared in the public schools of his home town in Maine where he was born September 25, 1885. George Smith thus brought with him to Yale not only an excellent experience in the fields of bacteriology and immunology, but a sound training at the primary and secondary levels. His "industry and loyalty" combined with "humor and patience" inspired the confidence and devotion of the students as well as of his colleagues.[3]

The academic years of 1917 and 1918 were hectic ones. World War I brought about the establishment by the Army of a training school for medical officers in the laboratory techniques of pathology and bacteriology in newly constructed "temporary barracks" which were not demolished until the addition to the "Brady" some ten years later, being in the meanwhile used as additional space for the Departments of Pathology and Surgery. In spite of this the teaching of students went on, as indicated by a letter from Dean Blumer that for the spring term of 1918 "there will be fifteen students taking the course in Bacteriology"—presumptively the first course given by Smith.[4] In the same letter it is apparent that the Dean had given him a committee assignment in respect to the organization of a medical library housed for the time being in the "Brady," various scattered collections being deposited there. A half-time librarian was to be engaged with an expected over-all budget of $1600. This was an assignment which lasted until 1945, during which time the library had consistently expanded and in 1940 had finally obtained its present facilities, in the design and organization of which he was intimately concerned.

This was his first initiation in academic administrative procedures and from then until his last illness he sat interminably upon committees, frequently as many as five at a time and both *ad hoc* and *ad infinitum*. The list is too long to record but gives evidence of a willingness and fidelity perhaps even beyond the call of duty. Of such are the annals of a faculty!

It was apparent that the new dean who took office in 1920 relied upon his colleague in the Department of Pathology, particularly in the early years of his regimen while the new faculty was in its formative period. So much was this the case that some disrespectful member of it parodied the title of a current "best seller" by quipping that, "If Winter comes, can Smith be far behind."

Recognition of his value to the school was not limited to the chores of administration. By 1923 the construction of the Sterling Hall of Medicine freed up space in the "Brady" and Smith's division was then given badly needed room on the third floor. His promotion to "Associate Professor of

[3] Minutes of the Board of Permanent Officers, Yale University School of Medicine, June 1951. Prepared by Professor Ira V. Hiscock on the retirement of George Hathorn Smith.

[4] Correspondence, etc. File of George Hathorn Smith, Office of the Dean, Yale University School of Medicine.

Bacteriology and Immunology" was concomitant with this and perhaps indicated a relative decrease of interest in the taxonomy of his field.

Three years later he was made Professor of Immunology, receiving also the honorary degree of Master of Arts from Yale. At this time and until 1942 "Bacteriology and Immunology" was a division of the University Department of Bacteriology, Pathology, and Public Health. In that year these three divisions were established separately as departments, the first becoming at a later date the Medical School Section of the University Department of Microbiology.

While the primary responsibility was from the beginning teaching and research, that of service to the New Haven Hospital was likewise important. In the first few years Smith carried an increasing load from that source, which was lightened when temporary laboratories of medicine and pediatrics were built for these disciplines and, somewhat later, the new surgical laboratory was established. This relieved bacteriology of a considerable routine load, but not of the serological tests for syphilis and the rapidly developing typing of blood for transfusions which eventually led to the establishment of a blood bank in the bacteriology laboratories. This development was of the greatest interest to Smith and it was fortunate that one so well versed in this particular field was at hand while this was in a somewhat experimental phase. It also provided blood and sera for further investigations.

Another routine responsibility, most successfully carried out, was the establishment around 1930 of a central sterilizing and media preparation laboratory providing service and economy for all the clinical departments. While Dean Winternitz initiated this, the organization and administration could not have been carried out without the willing and capable co-operation of Smith.

Surprisingly, with all these obligations, a very creditable amount of research was carried on in the department which, when published, did not always by any means bear the name of the chairman although inspiration and performance were alike provided by him. This has been well summarized by one of his later colleagues.

> Although in a number of scientific papers, in which he collaborated with his colleagues or students, Professor Smith turned his attention to a variety of problems, such as the action of staphylococcus toxin or the mechanism of the phagocytic process, the abiding loves, to which he ever returned, were the nature of the bacteriophage, and the serological individuality of each human being or animal. Time has shown how correct was his intuition as to the fundamental value of these systems for all biology.
>
> In a series of papers Smith explored various aspects of the bacteriophage problem, making distinct, original contributions. The achievement for which he is best known remains, however, his translation into English of Felix d'Herelle's books, *The Bacteriophage and its Behavior, The Bacteriophage and its Clinical Applications,* and the treatise, *Immunity in Natural Infectious Disease.* These translations not only stimulated much

interest and research in this new subject, but almost surely kept the flame of knowledge burning dimly but safely through the period of doubt and disinterest which preceded the recent brilliant revival of activity in bacteriophagy.[5]

His bibliography which is appended gives a measure not only of his scientific productivity, but also of his broad interest and competence in the literary field. Again quoting from his colleague:

> George Smith believed that experimental science was of little value to humanity if its data could not be effectively communicated, and he was interested in every detail of that communication process. Throughout his academic residence he was deeply interested in the library. . . . He was well acquainted with its resources in the history of medicine, and in addresses, such as those before the Beaumont Medical Club, he exhibited a marked skill at historical reconstruction, touched always by the deft phrase which revealed insight and humor. . . .
>
> Although always delighting in turning up, and brightening again, musty records of man's fight against disease, superstition and ignorance, George Smith was at his best in rendering into fit English the writings of scientists less gifted in expressing themselves. . . .
>
> The literary qualities which made Dr. Smith's essays and addresses such delightful reading are also found in the many book reviews which dot the pages of the Yale Journal. In a trenchant phrase he could bring merited praise, or deserved censure to an author. The range of material covered illustrates the catholicity of his interests. The review in which he summarized the reports of the medical experiments of the Nazis upon helpless captives in the word "Civilization!" justly deserves to rank with Heinrick Heine's single word characterization, a century before, of the censors of the German press.
>
> In addition to the scientific papers and translations already referred to, Smith also contributed chapters to works such as the George Blumer edition of the Billings-Forchheimer, *Therapeusis of Internal Diseases,* as well as a translation (with Dr. William B. Soper) of Calmette's treatise, *Tubercle Bacillus Infections and Tuberculosis.* He was also responsible for the indices to the volumes of the American Journal of Roentgenology for the years 1903 to 1937, and had hoped to bring these up to date if his health had permitted. At his death on July 7, 1952, he left a large file of material for a projected work on medical eponyms.
>
> The medical student always had a special place in George Smith's affections. Students in the classes he taught still recall vividly the personal greetings reserved for each, the special questions suited to the work at hand and, apart from the classroom, the friendly word of advice or encouragement which made each youngster feel that he counted. As Assistant Dean of the School of Medicine from 1940 to 1945 Doctor Smith had ample opportunity to exercise this interest in the student as a person, and many a useful citizen of the medical world today gratefully acknowledges his debt.

[5] Minutes of the Board of Permanent Officers, Yale University School of Medicine, October 1952. Prepared by Professor Henry P. Treffers following the death of George Hathorn Smith on July 7, 1952.

In the laboratory and in the seminar, Professor Smith's outstanding characteristic was that of ceaseless curiosity—of phenomena, people, causes and events. Although a gifted lecturer, he believed in teaching largely by questioning. In describing his philosophy of education in the address referred to above, he stated as the aim of his course at Yale: "Teaching, as the word is so often used, is reduced to a minimum, while the opportunity for the student to approach his work as a series of problems requiring a certain precision in methods of attack and clear thinking is extended to its maximum."

He also added: "A method highly successful at the hands of one teacher may be—frequently is—a failure in the hands of another."

It should appear on this record that Doctor Smith, although possessed of a highly developed and individual philosophy of teaching, believed in freedom of expression for the members of the department of which he was chairman for many years.

No picture of the man, even a solemn and sober acount, would be true and just if it omitted a reference to his spirit of humor, which was of a literate type rarely seen, and not always appreciated in this age. In speaking, for example, of courses in bacteriology (including his own) he wrote: "It is not my intent to argue that such courses are of necessity, wholly bad; in fact, were such courses better I might argue that they would be good." . . .

The tang of this remark is characteristic of his native state,[6] and he might well have been the inhabitant of it who, asked as to the mortality in his community, replied that he "guessed it was about 100%." Whatever he did he did intensively; even in his early years in New Haven while playing golf, by skirting the edges of the rough he came home with more balls than he started out with. After "signing off" this game, apparently because he thought it too seductive, he found great satisfaction in his summer vacations in Nova Scotia and a particular interest in his neighbors and the local mores. On leaving one year he left an order with a fisherman, he being also something of a carpenter, for a small rowboat. On returning the following year he was much pleased with the product but astonished at the low cost and queried the builder as to how he could afford to make so small a charge. The reply was that "he had some lumber lying around and he didn't have nothing else to do during the winter anyway." As Smith told this, he was tickled by the incongruous answer; of course each understood the other perfectly, but characteristically refrained from any emotional display.

Beneath the surface there was a great deal of feeling for those with whom he had pleasing contacts. It was not without significance that on the wall of his office were many portraits, not of distinguished individuals whom he knew, but of students who had worked with him and particularly of those who had been at one time or another on the editorial board of the Journal.

---

[6] An excellent example of this is to be found in the Winternitz number of this Journal (Smith, George H. A disciple of Hugh Quoil tarries briefly in New Haven. Yale J. Biol., 1950, *22*, 723.).

The conspicuous exception, as may be seen in the portrait painted of him at his desk in 1949, was that of Fred E. Pomeroy, Professor of Biology at Bates, under whom Smith studied as an undergraduate and from whom he received inspiration and encouragement then and throughout his subsequent career.

It was a peculiar satisfaction to both when Smith was given in 1934 the honorary degree of Doctor of Science by Bates College. At this he delivered an address, "The Philosophy of a Biologist" in which he referred to questions to which, as he said, science offers no answers.

> Matters which require faith, and since little can be gained by circumlocution, faith in God. Faith and science are not antithetic . . . full expression of abilities is to be obtained only when the individual is unhampered by too stringent restricting influences . . . an attitude of expectancy, a trust in natural forces operating through orderly progressive changes, should make us receptive to new ideas, tolerant and confident.

Curriculum Vitae

Born in Harrison, Maine, September 25, 1885.
B.A. Bates College, 1909.
A.M. Brown University, 1910.
Ph.D. Brown University, 1914.
A.M. (Hon.), Yale University, 1926.
Sc.D. (Hon.), Bates College, 1934.
Research Bacteriologist, H. K. Mulford Company, Philadelphia, 1912-17.
Assistant Professor of Bacteriology, Yale University School of Medicine, 1917-28.
Associate Professor of Bacteriology and Immunology, Yale University School of Medicine, 1923-26.
Professor of Immunology, Yale University School of Medicine, 1926-51.
Assistant Dean, Yale University School of Medicine, 1941-45.
Chairman, Yale University Department of Bacteriology, 1942-51.
Bacteriologist-in-Chief, New Haven Hospital and New Haven Dispensary, 1917-51.
Civilian Employee, Chemical Warfare Service, U.S.A., World War I.
Consultant, Medical Education, First Service Command, U.S.A., and Third Naval District, U.S.N., World War II.
Associate Editor and Editor, *Bacteriological Abstracts,* 1917-26.
Section Editor (*Immunology*), Biological Abstracts, 1927-40.
Editorial Board, *Journal of Bacteriology.*
Editor-in-Chief, *Yale Journal of Biology and Medicine,* 1928-49; Editor-in-Chief Emeritus, 1949-52.

Member, Medical Library Committee, Yale University School of Medicine, 1917, 1920, 1927-44; Chairman, 1917, 1932-41.

Member of the Beaumont Medical Club; President, 1943-44.

Member of the Advisory Board, Historical Library, Yale Medical Library, 1939-44.

Member of Library Committee, Yale University.

Member, Library Associates, Yale University.

Member of the Executive Committee, Division of Biological Sciences, Yale University.

Chairman and Director of Graduate Studies, Department of Bacteriology, The Graduate School, Yale University.

Member and Member of Executive Committee, National Board of Medical Examiners.

Chairman, Division of Bacteriology, National Board of Medical Examiners.

The George H. Smith Prize in Medical Microbiology

The George H. Smith Prize in Medical Microbiology was established in 1952 in honor of George Hathorn Smith, Ph.D., honorary M.A. 1926, a member of the faculty for thirty-five years and Professor of Immunology from 1926 to 1951. The prize is to be awarded annually to the second-year student in the School of Medicine who, in the opinion of the staff of the Department of Microbiology, has done outstanding work in the course.

BIBLIOGRAPHY OF GEORGE H. SMITH

Compiled by Emma M. Armonath

1911

The physiological effects of alkaloids of *Zygadenus intermedius*. Am. J. Physiol., 1911, *28*, 319-329 (with P. H. Mitchell).

1913

Size of the sample necessary for the accurate determination of the sanitary quality of shell oysters. Am. J. Pub. Health, 1913, *3*, 705.

1915

The production, through immunization, of specific ferments against bacteria as detected by the Abderhalden test. J. Infect. Dis., 1915, *16*, 313-318. Also: Boston Med. & Surg. J., 1915, *172*, 55.

The production and detection of specific ferments for the typhoid-coli group. J. Infect. Dis., 1915, *16*, 319-326. Also: Science, 1915, *n.s. 42*, 354.

1916

The mechanism of the Abderhalden reaction with bacterial substrates. J. Infect. Dis., 1916, *18*, 14-19 (with M. W. Cook).

Serum reaction following treatment with sensitized and non-sensitized bacteria. Med. Rec., 1916, *90*, 744.

The intracutaneous absorption of antigen. J. Immunol., 1916-17, *2*, 269-281 (with M. W. Cook).

1917

Cutaneous antibodies in the guinea-pig. J. Immunol., 1917, *2*, 415-420 (with M. W. Cook).

The absorption of antigen: a repetition of the work reported by Doerr and Peck. J. Immunol., 1917, *2*, 421-427 (with M. W. Cook).

1919

Intratracheal pulmonary irrigation. Contrib. Med. & Biol. Research . . . Sir William Osler, N. Y., 1919, *2*, 1255-1266 (with M. C. Winternitz).

1920

An unrecognized pathway for bacterial invasion of the respiratory tract. Abstr. Bacteriol., Balt., 1920, *4*, 17.

Effect of intrabronchial insufflation of acid. J. Exper. Med., 1920, *32*, 199-204 (with M. C. Winternitz and F. P. McNamara).

Epithelial proliferation following the intrabronchial insufflation of acid. J. Exper. Med., 1920, *32*, 205-209, 4 pl. (with M. C. Winternitz and F. P. McNamara).

Production of pulmonary infarcts by the insufflation of acid. J. Exper. Med., 1920, *32*, 211-217, 2 pl. (with M. C. Winternitz and F. P. McNamara).

An unrecognized pathway for bacterial invasion of the respiratory tract. Bull. Johns Hopkins Hosp., 1920, *31*, 63-66, 2 pl. (with M. C. Winternitz and E. S. Robinson). Also: Proc. Soc. Exper. Biol. & Med., 1919-20, *17*, 195.

1921

The precipitin test in the detection of *Bacterium diphtheriae*. J. Lab. & Clin. Med., 1921-22, *7*, 619-622 (with C. E. Kaufman).

1922

An allergic reaction to the tuberculous uterine horn. J. Immunol., 1922, *7*, 47-50.

Authorized translation of d'Herelle, F. *The Bacteriophage: Its Rôle in Immunity*. Baltimore, Williams & Wilkins, 1922, 287 pp., 14 illus.

1923

Authorized translation of Calmette, A. *Tubercle Bacillus Infection and Tuberculosis in Man and Animals*. Baltimore, Williams & Wilkins, 1923, 689 pp. (with W. B. Soper).

Serological factors of natural resistance in animals on a deficient diet. J. Immunol., 1923, *8*, 195-200 (with Isabel M. Wason).

1924

Authorized translation of d'Herelle, F. *Immunity in Natural Infectious Diseases.* Baltimore, Williams & Wilkins, 1924, 400 pp.

1926

The hypersensitiveness following injections of placental extracts. J. Immunol., 1926, *12*, 7-17 (with L. K. Musselman).

The hypersensitiveness following the administration of placental extract; the uterine horn reaction. J. Immunol., 1926, *12*, 31-33 (with L. K. Musselman).

The bactericidal action of serum following injections of adrenalin. J. Immunol., 1926, *12*, 205-218.

Authorized translation of d'Herelle, F. *Bacteriophage and Its Behavior.* Baltimore, Williams & Wilkins, 1926, 629 pp.

1928

Bacteriophage and phagocytosis; effect on resistant and dead bacteria. J. Immunol., 1928, *15*, 125-140.

1929

Bacteriophage therapy. In Blumer's edition of Billings-Forcheimer's *Therapeusis of Internal Diseases.* 1929 Supplement, pp. 27-44. New York, D. Appleton and Co.

The Calmette-Guerin Method (B-C-G) of Vaccination against Tuberculosis. In Blumer's edition of Billings-Forcheimer's *Therapeusis of Internal Diseases.* 1929 Supplement, pp. 130-137. New York, D. Appleton and Co.

1930

Protobacterial forms of *B. diphtheriae.* J. Bacteriol., 1930, *20*, 25-40 (with Elizabeth F. Jordan).

1931

Bacillus diphtheriae in its relationship to bacteriophage. J. Bacteriol., 1931, *21*, 78-88 (with Elizabeth F. Jordan).

Distribution of diphtheria bacteriophage. Yale J. Biol. & Med., 1931, *3*, 423-420 (with Elizabeth F. Jordan).

1932

Yale University School of Medicine, Division of Bacteriology. Methods and Problems of Medical Education. Rockefeller Foundation Reports, 20th Series, 1932, pp. 39-43.

1933

Hypersensitiveness and conditioned reflex. Yale J. Biol. & Med., 1933, *5*, 387-402 (with R. Salinger).

1936

Carcinolytic action of sera. Yale J. Biol. & Med., 1936, *8*, 603-618 (with Florence B. Mack).

1937

Carcinolytic action and erythrocyte sedimentation. Yale J. Biol. & Med., 1937, *9*, 173-184 (with Florence B. Mack).

Hypothetical A-factor and trypanosome infection. Yale J. Biol. & Med., 1937, *9*, 365-367 (with P. B. Cowles).

1938

Serologic patterns in human sera; hetero-agglutinins for sheep, mouse, and hen erythrocytes. Yale J. Biol. & Med., 1938, *10*, 391-404.

Serologic patterns of human sera; reciprocal hetero-agglutination reactions of human and sheep blood. Yale J. Biol. & Med., 1938, *10*, 449-454.

1939

Consolidated Indices—1902-1937: of the Transactions of the American Roentgen Ray Society, 1903-1908; American Quarterly of Roentgenology, Vols. I-V (1906-1913); American Journal of Roentgenology, Vols. I-IX (1913-1922); and Ameri-

can Journal of Roentgenology and Radium Therapy, Vols. X-XXXVIII (1923-1937). Springfield, Illinois, Charles C Thomas, 1939, ix + 451 pp.
Antigenic patterns of human sera; race-specific antigens. Yale J. Biol. & Med., 1939, *11*, 629-643.

1940

Some properties of staphylococcus culture filtrates. Yale J. Biol. & Med., 1940, *12*, 591-599.

1941

Thermostability of staphylococcus toxins. Yale J. Biol. & Med., 1941, *13*, 409-422.
Teaching of bacteriology. J. Asso. Am. Med. Coll., 1941, *16*, 153-157.

1942

Some physician friends of Joseph Farington, R. S.: I. Edward Jenner. Yale J. Biol. & Med., 1942, *14*, 307-332.
Some physician friends of Joseph Farington, R. S.: II. Anthony Carlisle. Yale J. Biol. & Med., 1942, *14*, 407-434.
Blood and blood substitutes for emergency use. Connecticut Med. J., 1942, *6*, 786-792.

1945

Consolidated Indices—1938-1942. Vol. II. Springfield, Illinois, Charles C Thomas, 1945.

1950

A disciple of Hugh Quoil tarries briefly in New Haven. Yale J. Biol. & Med., 1950, *22*, 723-726.

1951

Adrenal-hyaluronidase relationship in infection. Yale J. Biol. & Med., 1951, *23*, 361-369 (with Jeanette C. Opsahl).

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Eponyms: As applied in Bacteriology and Serology.
Not completed; manuscript deposited in Medical Library, Sterling Hall of Medicine, for completion by Emma M. Armonath.

* * *

This list of Dr. Smith's publications tells a story, but a very incomplete one, to the discerning person who actually reads the titles given. This person will realize that many of the publications of the past twenty-five years carrying the credit line, or by-line, "From the Department of Immunology, Yale University School of Medicine" could well, and most appropriately, be included here. However, Dr. Smith felt that students were entitled to sole authorship of all papers based on a Ph.D. dissertation or an M.D. thesis, and that the credit line, "From the Department of Immunology" showed his, as well as the Department's sponsorship of the student and of the subject. As a matter of fact, this might be said to be the policy of the Department, for during this period each student's report, no matter with which staff member the work might have been done, appeared as his own publication.

PHILOSOPHY OF A BIOLOGIST*

GEORGE H. SMITH

A quarter century is, indeed, a rather brief period to allow for the development of a philosopher, and yet, though I make no claim to such a title, I choose to preface the little I have to say by stating that it might be termed the "philosophy of a biologist." Perhaps I should also add that I can hardly qualify as a biologist, since my activities throughout the period in question have been confined to a very restricted aspect of biology. Thus, being neither a philosopher nor a biologist I am competent to discuss such a subject.

The past quarter-century has been—like every twenty-five-year period preceding it—the most remarkable one in history. Not only is this true as regards the sciences and the progress made along innumerable lines, but is equally true of other fields which bear more or less directly on everyday life. Both evolution and revolution have had their innings. We have endured, and some have survived, the "war to end wars"; we have experienced the "noble experiment" and have profited thereby if by chance we belong to certain classes of society; and more recently we have witnessed a mobilization of alphabetic characters into groups, large or small, to be applied like porous plasters to the ills of segments of our suffering humanity. Great changes have occurred, and yet it is difficult to avoid the suspicion that mankind remains much the same, with the same basic traits and the same fundamental needs; with the same biologic constitution finding expression through the same native mechanisms. The picture is far from being throughout a drab and dull one, but the difficulty in translating ideals into motivating ideas gives one pause. Possibly an explanation, in part at least, for this failure is failure to grasp the inherent nature of the material operated upon, and operating. Basically, man is a biologic system, in more or less conflict with its environment; and generally speaking it is the environment which modifies the system, rather than the reverse. Evolutionary processes are as inexorable as they are subtle, and are conditioned by forces operating upon and through innate attributes, not through organizing a "committee" and adopting a slogan. But such thoughts as these express, of course, nothing new; indeed, they are obsolete and unworthy, lacking all appeal to the "man with a mission" who is determined to make the best of the "best of all possible worlds" without further ado.

The only justification for restating such unorthodox beliefs is to be found in a consideration of the changes wrought in the field of the sciences

* Delivered at Bates College in 1934, when Bates conferred upon Dr. Smith the honorary degree of Doctor of Science.

during this period of economic and political experimentation. We find that science has its vagaries too, and if we enquire closely into current beliefs, we find but little remaining that held our faith but twenty-five years ago. It is needless to enumerate the astounding changes made, but it may be pertinent to point out the nature of those changes, even though we may deplore them. To the casual observer all of these newer developments partake of the miraculous, and, like such poorly understood phenomena, seem worthy of admiration. But to get to the greatest number of places where, all too frequently, one is neither needed nor wanted, in the shortest space of time is in itself hardly sufficient to command reverence. To have developed contrivances that permit leisure without coincidentally elaborating an art of being gracefully lazy entails its own penalties. It is doubtful if the time will ever come when a toot on the horn, however vigorous, will compensate for judgment. Such are some of the results of advances which, on the whole, are salutary. But the point worthy of emphasis is that these scientific developments have evolved through a method of serial increments. The evolution of the machine is just as certain—perhaps even more certain than is the evolution of the biologic system, a gradual process.

Such ideas may seem out of place in what has been termed the philosophy of a biologist, but I would say that they have evolved from a consideration of what has transpired in my own little field of activity during the last few decades. When I entered this field—bacteriology—twenty-five years ago it offered a most satisfying prospect. One had the bacteria, properly cataloged and named, one had the environment, man or otherwise. What more simple than to place the two in juxtaposition, observe the result, and for want of a better thing to do, write a paper about it. One other thing was available—a set of fixed and all-inclusive laws governing biological systems. It was inconceivable that a bacterium, however recalcitrant, should violate laws set and established. Laws governing morphological limitations, defining processes of assimilation and reproduction, and specifying definite responses to external stimulation were held to be final, applicable to the microcosmos as well as to the world of larger beings.

Today the situation is changed; the general application of so-called biologic laws is questioned, and it is recognized that they are valid for specific cases only. In the microcosmos they have been abrogated, and for them has been substituted an entirely new set of dicta, denominated laws, the outgrowth of gradually increasing knowledge of the beings concerned. We now know that the character of a bacterium is not fixed and immutable; we know that it may exist in a diversity of forms and with a wide divergency of attributes, both morphological and physiological. At one time the organism may exhibit characters which entitle it to the term cell—that indivisible unit of living matter—and at another time that same organism may have so fragmented that the most powerful of microscopes will fail

entirely to reveal its presence. And yet, in this nebulous state the spark of life is still retained, indeed, is present in each of the multitude of fragments, from each of which a new organism, conserving more or less unmodified the properties of the original, may develop. Is the bacterium a cell? Is the cell the unit of life? Does life require certain structural conformations and spacial relationships? Is the law at fault, or is the bacterium delinquent?

Again, reproduction in the microcosmos has been said to be of the most elementary type imaginable, simple fission. While beyond question fission does occur, thus giving origin to two organisms from the parent being, is it certain that this process is as simple as it sounds? Can we say with assurance that it is the only method operative? When one considers that out of a population of millions all arising from a single being, no two are endowed with the same mosaic of characters, and that in this reproductive process character segregation takes place, one wonders if it is the fission or the observer that is simple. Life is not organized to meet the limitations of the microscope, however convenient this would be to the formulator of laws.

If we pass to that class of beings, most intangible of all, the filter-passing viruses, faith is subjected to further strains. Invisible, imponderable, and yet with clearly defined properties, these beings violate all laws, chemical and physical, as well as biological.

Protoplasm, which in its essence offers sanctuary to life, has been held to be constituted of definite components, such as that particular arrangement of elements termed the protein. But what shall we say when it appears that some of these fully equipped beings are smaller than is the protein molecule? What must we conclude when we find that for such beings *all* laws, even the law of gravity itself, seem to be suspended?

True, one need not be too greatly disturbed at this seeming lack of order, unless perchance faith in a regulator of all things is lacking. Indeed, if one adopts the attitude of the paternalistic type of government, it is quite possible to hold the natural tendencies in abeyance, to reduce variability to a minimum, and to standardize an entire bacterial population at the level of the moron. But left to themselves, each to carry on its conflict with a constantly changing environment, individuality and initiative become apparent, for which no cause can at present be ascribed.

What form, then, should the philosophy of a biologist assume? To what can he pin his faith? First, that despite the oft-quoted axiom that science is teaching us more and more about less and less, there are questions to which science offers no answers; matters which require faith, and since little can be gained by circumlocution, faith in God. Faith and science are not antithetic. Second, that however sound may be our beliefs today, another day and generation will regard them false, and will, we hope, be tolerant. Finality is not our prerogative. Third, that a full expression of abilities is

to be obtained only when the individual is unhampered by too stringent restricting influences. Standardization and progress are not synonyms. Fourth, that an attitude of expectancy, a trust in natural forces operating through orderly progressive changes, should make us receptive to new ideas, tolerant and confident. These things may all be learned from a study of the world of diminutive beings; and may, if you choose, constitute a philosophy of a biologist.

LYSOGENESIS IN BACILLUS MEGATHERIUM*†

PHILIP B. COWLES

That bacterial cultures can be chronically infected with viruses has been recognized almost as long as the bacteriophage itself has been known. Lysogenicity, as this host-parasite relationship is commonly called, has been observed with almost every bacterial species in which virus infection has been studied. Two instances will serve to call attention to the fact that infection of the bacterial cell is associated with, if, indeed, it does not cause, important changes in the host. These changes may well have significant impacts upon man, for, following Smith and Jordan's[11] early observation that all of the field cultures of *C. diphtheriae* which they examined carried bacteriophage, Freeman[6] and Freeman and Morse[7] have found that the ability of this organism to produce toxin is always accompanied by infection of the bacterial cell by phage, and that when the cell loses its virus, it also loses its toxigenic power. This relation is probably one example of a broader phenomenon described by Zinder and Lederberg,[13] a phenomenon, termed "transduction," in which it has been shown that infection of one strain of an organism with phage from another lysogenic strain is associated with a transfer of genetic characteristics, and the transferring agent seems to be identical with the virus. These two citations should suffice to suggest that the phenomenon of lysogenicity may have far-reaching implications.

While both of these examples have been drawn from the bacteria, the study of the lysogenic process itself has been most profitable with the spore-forming bacilli, and much of the attention has been directed towards *B. megatherium*. A review by Price[10] summarizes most of the pertinent information, and more recently papers by Baer and Kruger[1] have described studies on *B. mycoides* and its phage. In this paper will be presented some observations which have been made on several strains of *B. megatherium* and some of its viruses.

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* From the Department of Microbiology, Yale University. Aided in part by grants from the Fluid Research Funds of the Yale University School of Medicine.

† Although in his original description of this organism de Bary spelled the name *megaterium* (big rod), this has always been assumed to have been a typographical error, and the spelling *megatherium* (big animal) has commonly been used.[2] Currently, however, the original spelling is being employed. Anyone acquainted with this big bacillus who also has seen a picture of reconstructed Megatherium—that gigantic, extinct, sloth-like edentate—will sympathize with the author's preference for customary usage, although the first printing of the name, right or wrong, undoubtedly has priority.

Received for publication March 10, 1953.

Materials and Methods

The strains used were the following:

899 received from den Dooren de Jong in 1930.

S an asporogenous and highly sensitive strain received from Hershey as PR3E.

C, S9a, S9b isolated by the author from various soils* and characterized only from morphology and phage susceptibility.

The bacteriophages employed:

M1 isolated from culture 899.

M2, M3, M4, M5 isolated from soils and described in a forthcoming paper by Friedman and Cowles.

M7 another virus isolated from soil, but as yet unstudied.

The broth medium routinely used contained 12 g. of Bacto yeast extract and 4 g. of Trypticase per liter at pH 7.4. For plating purposes the agar layer technique was used. The basic medium for this was made by adding 15 g. of agar to each liter of broth, or 7 g. for the semi-solid agar used to pour the supernatant layer containing the inoculum. An agar containing only 25% of the two nutrients was used for slants to promote sporulation, and is referred to as "qs" agar. All cultures were grown at 30° C.

In order to grow cultures continuously through many generations, a culture vessel was installed in a shaker placed in an electric refrigerator fitted with an auxiliary thermoregulator. A phototube and its accompanying circuit kept the culture density within the desired limits, adding fresh medium when needed. The surplus flowed into a waste vessel, so that the volume remained constant.

The Production of Lysogenic Cultures

The simplest method of obtaining lysogenic cultures has been found to be the inoculation of qs agar slants from broth cultures which had been growing for a day or so in the presence of the desired phage. With most strains of *B. megatherium* an abundance of spores will develop on these slants after a day or more of incubation at 30° C. These spores are transferred to broth, heated at 80° for ten minutes to kill both vegetative cells and free bacteriophage, and then, in dilution, plated with and without the indicator culture, routinely culture S. After incubation, a colony growing in the center of a plaque is picked to a fresh qs slant, and the spores developing in the growth on this slant, after heating, serve as the start of the lysogenic culture. Several of these will be described, all obtained with strain C by infecting subcultures of this strain with the six M phages mentioned above, as these newly formed lysogenic cultures seem to fall into two main classes, called, for convenience, Group A and Group B. The phages, too, are grouped according to their lysogenic cultures.

For simplicity in referring to such cultures, the designation of the strain of *B. megatherium* will be given first, followed by the phage number in

* Many of the soil samples were made available through the kindness of Dr. P. R. Burkholder, who had collected them from various parts of this country and Mexico as a source of organisms producing antibiotics.

parentheses. For example, strain C infected with phage M7 will be written C(7), and the "standard" lysogenic culture 899 will be called 899 (1).

Of the several lysogenic C strains, C(1) and C(5) were similar to 899(1) and have been placed in Group A. When heated spores of any of these three lysogenic cultures are plated out against indicator S, and also

TABLE 1

REPRESENTATIVE COUNTS OBTAINED FROM HEATED SPORES OF LYSOGENIC CULTURES PLATED ALONE (A) AND WITH INDICATOR CULTURE S (B). PLATES OF 10^{-4} DILUTIONS

	Group A			Group B		
	899(1)	C(1)	C(5)	C(2)	C(3)	C(7)
(a) Colonies	40	82	263	191	130	63
(b) Plaques with center colonies	42	71	200	7	8	2
Clear plaques	2	2	3	39	48	10

TABLE 2

GROUP A. COUNTS MADE FROM PLATES INOCULATED WITH 10^{-5} DILUTIONS OF C(5) GROWING IN A CONTINUOUS SHAKE CULTURE STARTED FROM HEATED SPORES

Day of culture	*Approximate generation*	*Colonies*	*Plaques with center colonies*	*Clear plaques*
1	1	35	31	140
2	40	117	130	290
3	92	88	89	145
4	150	32	15	181
5	187	86	79	140
6	225	151	116	285
7	264	116	95	117
8	294	237	200	310

Inoculation of a qs slant on the last day yielded a culture apparently identical with that used for the initial inoculum.

alone, for colony counts, the number of colonies developing on plates seeded only with spores is essentially the same as the number of plaques on the indicator plate. Furthermore, almost every plaque contains a center colony which, upon subculture, yields a lysogenic strain. Apparently, with these three cultures the host-parasite relationship is well established, and the great majority of spores and cells are lysophoric, passing this property on to the cells developing from them. As has been demonstrated with 899, only occasional cells appear to become resistant, or susceptible and lysogenic.[4, 8] By "lysophoric" is meant the ability to carry and transmit the precursor of bacteriophage,[8] while "lysogenic" implies the actual production and libera-

tion of active and detectable virus. Hence a culture can be both lysophoric and lysogenic, but a cell, when it becomes lysogenic, is lost.

The other four lysogenic cultures, C(2), C(3), C(4), and C(7), behave in a different manner and have been put into Group B. These cultures, as such, always carry their respective viruses, at least when grown under standard conditions, but the spores and component cells display a varied pattern. Many of the plaques developing from heated spores seeded on indicator plates fail to show any center colony, in some cases, on countable plates, all of them. Moreover, the number of plaques with and without center colonies is usually less than the number of colonies on the comparable plates

TABLE 3

GROUP B. COUNTS MADE FROM PLATES INOCULATED WITH 10^{-5} DILUTIONS OF C(7) GROWING IN A CONTINUOUS SHAKE CULTURE STARTED FROM HEATED SPORES

Day of culture	*Colonies*	*Plaques with center colonies*	*Clear plaques*
1	410*	0	134
2	105	0	71
3	139	2	38
4	112	0	36
5	197	0	2270*
6	600*	0	320*

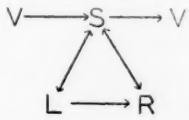
* Counts calculated from plates of other dilutions.

inoculated with heated spores only. If agar seeded with indicator culture is poured over this latter plate after the colonies have developed, plaques will be formed where no colony had been seen, and some of the colonies obviously have released no phage, apparently being composed of resistant cells. In Group B, then, the host-virus relationship seems to be different from that found in members of Group A. Not all spores are lysophoric; furthermore, some of those that do have this property seem to yield progeny that lyse rather quickly before any detectable colony has been formed. Obviously, microscopic observation of germinating spores is needed to clarify this point. And yet, in the gross the cultures appear stable, and several have been carried through over a hundred transfers, and for more than a year, without any alteration of their appearance, but with the same continuing, erratic behavior of their individual elements. The tables present typical data obtained with cultures of each group.

It is a simple matter to obtain, from any member of Group B, strains of bacteria which are resistant and virus-free. Such strains, however, readily become lysogenic when subsequently cultivated with virus. The results of experiments make it seem probable that most, if not all, cultures which are resistant to phage, but not lysophoric, and which do not adsorb phage

detectably, do, when cultivated for many generations in the presence of the virus, become lysogenic. In connection with this point it is rewarding to re-read the section on mixed cultures in d'Herelle's classic.[8]

In general, perhaps, the condition in a culture containing both bacteria and virus may conform to the following scheme, where V = bacteriophage, S = susceptible cells, R = resistant cells, and L = lysophoric cells or spores:



In an actively growing lysogenic culture there is normally maintained an equilibrium which is characteristic of that culture, determined in part, probably, by factors in the cell and in the virus. Changes in the physical and in the chemical environment can undoubtedly affect this equilibrium also, as Lwoff *et al.*,[9] Welshimer,[12] Ehrlich and Watson,[5] and Clarke[3] have shown. At one end the limit would seem to be reached when all cells stay in, or are converted to, the susceptible state (culture S is normally an example of this), and the result is the loss of the culture. The other end would be approached when the frequency of changes to susceptibility is so low that no such forms occur in the number of cells commonly reached in a growing culture. Here it would be difficult to determine whether or not a culture was, in fact, lysogenic, although obviously the nature of the indicator strain is important. Of the two groups of cultures described above, Group A represents one in which the equilibrium lies in the direction of L, while in Group B the shift is predominantly towards R.

The bacteriophages mentioned here are only a few of some dozens which have been isolated against indicator S from soil samples. From the same samples in which the viruses were found, strains of *B. megatherium* (as yet characterized only on the basis of morphology) have also been obtained. Apparently in most soils containing phage, both susceptible and resistant organisms are present. For example, the soil yielding phage M2 gave culture S9a, susceptible, and S9b, resistant. No lysogenic strain was found. Both S9a and S9b were susceptible to other phages, and each could be made lysogenic for M2. It seems highly probable that a few lysophoric cells in the soil were the sources not only of the phages recovered, but also of the susceptible and of the resistant cultures. In the laboratory-induced lysogenic cultures of Group B, only a small percentage of spores are lysophoric, and it is pure chance if one selects a lysogenic colony from all of those developing on a plate seeded with heated spores only. Reference to C(2) in Table 1 will make this clear, for apparently only 7 out of 191 spores carried the potential virus. In heated soil samples, containing many spores of other species, the probability of finding a lysogenic culture of the species sought is

obviously slight. At any rate, none was found in any of the soils tested. If one were to hazard a guess, it would be that the natural *B. megatherium* 899(1), as well as the C(1) and C(5) strains developed here, are types of association occurring less frequently in nature than those represented by the members of Group B. Certainly, the isolation of naturally occurring lysogenic cultures has not frequently been reported in the literature.

It is, of course, impossible to say which type of association—that found in Group A or that present in Group B—is better for the host and which more favorable for the virus. The two Group A phages, M1 and M5, seem to be tolerated by most of their hosts' cells, but they appear to be of rather weak virulence and form small plaques or very turbid ones. On the other hand, the Group B viruses, M2, M3, M4, and M7, are more virulent and form large plaques over a wide host range. But they seem less able to establish a symbiotic relationship with the host's cells, and most of the latter develop a resistance to the virus. The B phages, nevertheless, are quite able to perpetuate themselves. In our own society we might feel that the characteristics of Group A viruses are found in individuals with an interest in security, while those of Group B go with a desire for free enterprise.

Before concluding, it is impossible to refrain from speculating on the possibility that equilibria similar to those described above may play some part in man's immunity following virus infections, particularly in those diseases in which the immune state is postulated to depend on the persistence of the latent virus. When an individual recovers from the initial assault of the invader (and the reader may throughout this argument interpolate the part played by antibodies), most of his once-susceptible cells would become resistant and the rest virophoric. A few of the latter would at intervals become virogenic and release virus. There would be concomitantly a slow reversion to susceptibility of some members of the resistant population of cells. These new susceptible cells would be invaded by the free virus, with the death of some and the conversion of others to the virophoric state. This delicate balance might persist for life, and the individual be immune. Should conditions bring about a more rapid development of susceptible cells, a flare-up of the disease might ensue (Brill's disease, "cold sores"). On the other hand, if more virus were released in the immune individual than his susceptible cells could handle, it might spill over and make him a carrier in the usual sense of the word, and the starting point, perhaps, for an epidemic. Such a process could explain the appearance of a virus disease in the absence of any recognized case. Finally, if the virus should die out in the virophoric cells, the remaining resistant cells and their descendants, lacking the stimulus of the virus, might revert entirely to susceptibility, and the individual's immunity would be lost.

Summary

1. The behavior patterns of six viruses, all parasites of *B. megatherium*, are described and shown to fall into two main groups.

2. Group A consists of two viruses, M1 and M5, having a low degree of virulence, but able to parasitize most of the host cells. Group B viruses, M2, M3, M4, and M7, are much more active, but can establish a symbiotic relationship with only a few cells in a population, and that transiently. They can, none the less, maintain their existence.

3. Some discussion is essayed of the lysogenic, or lysophoric, state as it occurs in nature, and of the possible prevalence of a similar parasitism in man.

References

1 Baer, S. and Krueger, A. P.: The *B. mycoides* N host-virus system. J. Gen. Physiol., 1952, *35*, 471.

2 *Bergey's manual of determinative bacteriology.* Breed, R. S., Murray, E. G. D., and Hitchens, A. Parker, Eds. Sixth ed. Baltimore, 1948, p. 714.

3 Clarke, N. A.: Studies on the host-virus relationship in a lysogenic strain of *B. megaterium*, II. J. Bact., Balt., 1952, *63*, 187.

4 Clarke, N. A. and Cowles, P. B.: Studies on the host-virus relationship in a lysogenic strain of *B. megaterium*. J. Bact., Balt., 1952, *63*, 177.

5 Ehrlich, H. L. and Watson, D. W.: Lysogenesis of *B. megatherium*. J. Bact., Balt., 1949, *58*, 627.

6 Freeman, V. J.: Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*. J. Bact., Balt., 1951, *61*, 675.

7 Freeman, V. J. and Morse, I. U.: Further observations on the change to virulence of bacteriophage-infected avirulent strains of *Corynebacterium diphtheriae*. J. Bact., Balt., 1952, *63*, 407.

8 d'Herelle, F.: The bacteriophage and its behavior (Smith, G. H., Tr.) Baltimore, 1926.

9 Lwoff, A., Siminovitch, L. and Kjeldgaard, N.: Induction de la production de bactériophages chez une bactérie lysogène. Ann. Inst. Pasteur, Par., 1950, *79*, 815.

10 Price, W. H.: Bacterial viruses. Annual Rev. Microbiol., 1952, *6*, 333.

11 Smith, G. H. and Jordan, E. F.: *Bacillus diphtheriae* in its relationship to bacteriophage. J. Bact., Balt., 1931, *21*, 75.

12 Welshimer, H. J.: Observations on phage output and on anaerobic lysis of lysogenic strains of *B. megatherium*. J. Bact., Balt., 1951, *61*, 153.

13 Zinder, N. D. and Lederberg, J.: Genetic exchange in *Salmonella*. Bact. Proc., 1952, 43.

THE SYNTHESIS OF PROTEIN-AZOBENZYL-NUCLEIC ACID COMPOUNDS*

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Nucleic acids are found in all living cells in amounts which might appear to confer on them a rôle as structural constituents. Nucleic acids have also been associated with the activities of genes and bacterial transforming factors, particularly for the pneumococcus. The specificities of the changes mediated by them suggest strongly that the nucleic acids themselves vary in some characteristic structures. Although an important beginning has been made in the chemical attack on nucleic acid composition which may result in some elucidation of the problem, our present knowledge is still quite incomplete.[1]

Since the current situation parallels in many respects that for protein and polysaccharide structures a generation ago, it appeared that the same methods which proved so helpful in demonstrating the individualities of these substances could be used for nucleic acids. Prominent among these are, of course, serological techniques. While admittedly these do not, in their present state of development, permit assignment of definite structures to antigens, they can nevertheless permit of clear distinctions among certain structures, and varying degrees of relationships can be assigned with some quantitative precision.

An immediate difficulty in the application of serological techniques to the present problem is apparent, however, in that although nucleic acids form a part of numerous antigens, including viruses, there is as yet no evidence that any portion of the specificity of the resulting antibodies is directed against the nucleic acid component. This is illustrated by the general experience that isolated nucleic acids cannot inhibit the reaction of nucleoproteins with antibody—a test which with other systems has proven to be a most delicate indicator of serological relationships. Even though the nucleic acid may not influence the *character* of the antibody, there is evidence that for at least one antigen—the turnip yellow virus—the presence of nucleic acid can influence materially the *amount* of antibody directed toward the protein component.[2]

In our analysis of the failure of nucleic acid to function as a hapten it appeared that perhaps its linkage in the usual nucleoprotein was inappro-

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Received for publication March 16, 1953.

priate for the exertion of an antigenic function (as an extreme instance, it has been postulated that in the turnip yellow virus the nucleic acid is inside of the protein shell[6]) and that some success might be achieved with another type of linkage to protein.

The present paper reports the results of our attempts to link some nucleic acids to proteins. Apart from serological systems, the preparation of such complexes may have applicability to other branches of nucleic acid study. The details, particularly the precautions as to the homogeneity of the final product or the state of aggregation of the nucleic acid component, will have to be adjusted to the particular requirements of each problem. Although a refined serological analysis would require an antigen of a high degree of homogeneity, this was felt to be an unnecessary restriction in the present work, since the answer desired was of the all-or-none variety, and a high degree of control over the specificity of the reaction could be exercised through the use of a purified, relatively undegraded inhibiting antigen rather than an immunizing coupled antigen of this character.

Methods and Materials

It was desired to form stable nucleic acid-protein complexes which would not be dissociated by relatively mild chemical agents, such as 1M sodium chloride, or be split readily by normal mammalian enzymes, and which might thus be expected to persist *in vivo* for reasonable periods of time. A method was desired which would be applicable to a wide range of nucleic acids and proteins.

It was believed that the plan followed by Goebel and Avery[2] to couple pneumococcal polysaccharide to protein would be applicable to this problem. This proved to be the case. This paper reports the preparation of several combinations of desoxyribosenucleic acid (DNA) and protein by methods similar to those used by Goebel and Avery. Nucleic acid preparations used included: (a) a relatively non-polymerized commercial material derived from fish sperm and testis, (b) a polymerized material prepared from calf thymus by the method of Gulland, Jordan, and Threlfall,[8] (c) a somewhat degraded preparation from a rapidly growing mouse carcinoma. Proteins used included: (a) crystalline bovine serum albumin, (b) various streptococcal fractions, including the M substance.

Coupling was accomplished in three stages:

a. introduction of a nitrobenzyl group into the DNA
b. reduction of the "nitrobenzyl DNA" to "aminobenzyl DNA"
c. diazotization of aminobenzyl DNA and coupling to protein

1. *Preparation of nitrobenzyl DNA.* DNA was dissolved in water and sufficient dilute sodium hydroxide to yield a neutral solution containing ½% to 4% DNA. Crystals of p-nitrobenzyl bromide were added, usually in amount equal to one-half the weight of DNA used. This proved to be a significant excess. After the mixture was placed in a boiling water bath and stirred rapidly, the crystals melted and became emulsified with the DNA solution. The pH of the reaction mixture was determined frequently with pH paper, and 1N sodium hydroxide added dropwise to maintain a slightly alkaline pH. The reaction was terminated when a persistent pH of 9 was obtained. This generally required 30 to 50 minutes. This reaction mixture was now turbid, and the odor of nitrobenzyl alcohol could be noted. The mixture was then poured into two volumes of acid ethyl alcohol (final pH, 2-3) and placed in the cold

room overnight. The following day the mixture was centrifuged and the supernatant solution discarded. The precipitate was washed with acetone and then twice with ether and dried in a vacuum desiccator.

Nitrobenzyl DNA was obtained as a white to tan powder in a yield of approximately 75%. Upon neutralization it could be dissolved in water, but the solubility was distinctly lower than that of the original DNA. Absorption in the ultraviolet was more intense than that of DNA, on a weight basis, and the shape of the absorption spectrum was changed, with a shift of the maximum toward 270 mμ and significant absorption beyond 310 mμ. The curve obtained (Fig. 1) could not be a summation of curves for DNA and nitrobenzyl bromide, although it was not strikingly different from the expected absorption of a 4:1 mixture of the two.

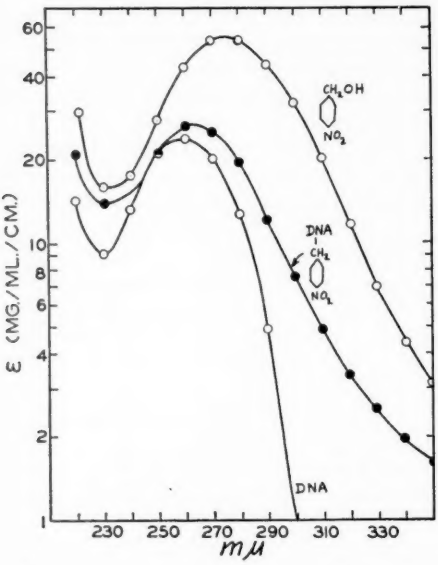

FIG. 1. Absorption spectrum (on semi-log plot) of p-nitrobenzyl DNA, with comparison spectra of original DNA and p-nitrobenzyl alcohol.

2. Preparation of aminobenzyl DNA. Nitrobenzyl DNA was suspended in 25 to 100 volumes of water and neutralized with 1N sodium hydroxide. The suspension was warmed to 50° C. and maintained between 48° and 55° during the reduction. It was stirred continuously while a freshly prepared saturated solution of sodium hydrosulfite was added in small increments. The mixture was maintained near neutrality by addition of 1N sodium hydroxide as required. The reaction was continued until an excess of the reducing agent persisted, as determined by the ability of the reaction mixture to bleach a dilute solution of methylene blue. As the reduction approached completion, the mixture cleared, and an opalescent, yellowish to golden solution was produced. This was poured into two volumes of acid alcohol (final pH 2-3), producing a flocculent precipitate of aminobenzyl DNA, contaminated by free sulfur and some sulfite. The precipitate was collected by centrifugation and washed. It was then dissolved in water, with the addition of sufficient 1N sodium hydroxide to bring the solution to neutrality. This solution was centrifuged, the sediment discarded, and the supernatant solution filtered through a thick retentive filter paper. Much of the sulfur was eliminated by this procedure, and a clear, yellowish to golden solution was obtained. A second precipitation in acid alcohol, followed by acetone and ether washes and drying in a vacuum desiccator, was used to obtain a dry material for weighing and storage. The yield at this stage was again approximately 75%.

Aminobenzyl DNA had a distinctly higher solubility in water than nitrobenzyl DNA. Compared to nitrobenzyl DNA its ultraviolet absorption spectrum showed a shift

toward the shorter wave lengths, with a maximum at 260 mμ, and less absorption beyond 310 mμ.

3. *Diazotization of aminobenzyl DNA and coupling to protein.* Diazotization of aminobenzyl DNA was accomplished in acetic acid at 0° C. Sufficient 0.1N sodium nitrite was added to produce a slight but distinct excess, as indicated by a positive reaction with starch-iodide paper. The reaction mixture was stirred for one-half to one hour, cautiously neutralized, and poured into a solution of the selected protein in dilute sodium carbonate. The amounts of protein and diazotised aminobenzyl DNA were usually adjusted so that a slight excess of free diazonium was present after one hour. This was tested for by adding a few drops of the reaction mixture to α-naphthol in sodium carbonate. An orange color usually developed within two or three minutes of the start of the coupling reaction.

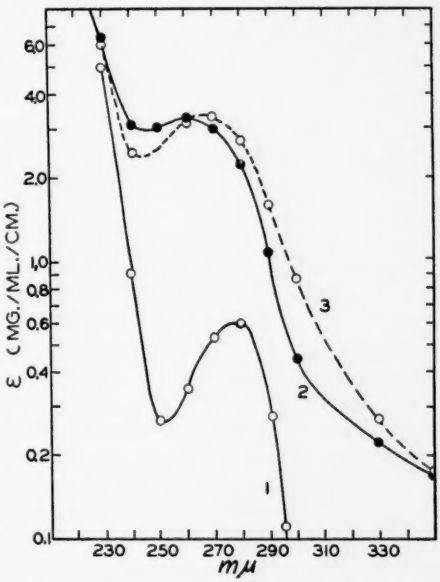

FIG. 2. Absorption spectra (on semi-log plot). Curve 1: bovine serum albumin used; curve 2: albumin-azobenzyl-DNA; curve 3: calculated values for albumin and p-nitrobenzyl DNA mixture of ratio 8:1.

The method of separation of the coupled product from other constituents of the reaction mixture varied with the protein used. When bovine serum albumin was the protein, separation was accomplished (at 4° C.) as follows:

a. The reaction mixture was brought to 67% ethyl alcohol and pH 8-9. Under these conditions the coupled compound precipitated almost quantitatively, but uncombined albumin and part of the uncombined DNA remained in the supernatant solution. The precipitate was collected by centrifugation and dissolved in water.

b. The solution thus obtained was brought to 40% alcohol and pH 4.5 (with acetic acid). Under these conditions the coupled compound again precipitated, but the benzylnucleic acid compounds did not.

The final product, protein-azobenzyl-nucleic acid, was usually yellowish to orange in color, dissolved to yield clear orange solutions, and behaved as a protein similar to the parent substance. The effect of the introduced groupings was, however, readily seen in such characteristics as isoelectric point, ultraviolet absorption, etc. One of the compounds studied in some detail was albumin-azobenzyl-desoxyribosenucleic acid.

This compound was prepared from a commercial DNA (Schwarz) and crystalline bovine serum albumin. It was orange and yielded a clear orange aqueous solution which became yellow on dilution. The phosphorus content was 1.11% by weight, and nitrogen was 13.3%. This corresponds to a ratio of protein: azobenzyl DNA of 6:1. The ultra-

violet absorption is shown in Figure 2, together with the spectrum of albumin alone, and for comparison, a curve constructed for a hypothetical compound of albumin and nitrobenzyl DNA in the ratio of 8:1. The latter value gives the closest fit to the experimental curve; exact correspondence is not to be expected since the compound actually synthesized contained a linked azo instead of a terminal nitro group.

Results of electrophoresis were as follows:

a. At pH 4, in 0.2N acetate buffer the compound moved slowly toward the negative pole. Only one component was seen. Movement of the yellow color coincided with movement of the electrophoretic component.

b. At pH 4.9 in 0.2N acetate buffer the compound moved very slowly (2 cm. in 4 hours) toward the positive pole. A sharp peak was seen together with a very small, more rapidly moving component.

c. At pH 7.6 in 0.05N borate buffer movement was at a rate of approximately 1.5 cm./hr. A sharp boundary was seen, but this was not quite as good as that in run (b) above. A very small, more rapidly moving component was seen.

Discussion

The objective of the synthesis described in this paper was the preparation of an antigenic complex in which the nucleic acid portion, in contrast to that of native nucleoproteins, would function as a hapten and thus permit comparisons of the specificities of nucleic acids from various sources. There is no doubt that a compound has been prepared which contains protein and nucleic acid linked through an azobenzyl intermediate. Satisfactory agreement is obtained between estimates of its composition based on phosphorus content and on analysis of absorption spectra. It can be assumed that the bonds with the protein are of the type usually obtained in a diazo coupling.[4] The precise nature of the combination between nitrobenzyl bromide and DNA has not been determined in the experiments reported here. Further study may be expected to yield more definite information on this point.

In the one synthesis where a polymerized, viscous sample of thymus DNA was used, the viscosity had disappeared by the time the combination with nitrobenzyl bromide was completed. Whether this represented depolymerization of the DNA, or some other change, is not certain. Certainly, depolymerization might have been expected under the conditions of the reaction. This was not necessarily disadvantageous so far as the ultimate objectives of this investigation are concerned. However, future work is planned in which coupling will be attempted under milder conditions.

The serological aspects of this study, which are still incomplete, will be reported in detail elsewhere. It may be said in brief that although these complexes do induce antibody formation in rabbits, no haptenic activity has as yet been demonstrated for the nucleic acid component. However, there is an ample body of experience with other haptens which indicates that the results of even a moderate number of negative trials cannot be taken as conclusive, since positive haptenic effects may appear, in unpredictable

fashion, only with arbitrary protein combinations, and then only with sera from certain animals.[5]

In addition to their utility for serological studies, the compounds herein described may find application in investigations of the infectivity of viruses or the enzymatic degradation of nucleic acids. For some of these purposes it will undoubtedly be necessary that the nucleic acid component retain as nearly as possible its original degree of polymerization, and for this end coupling procedures will be required which are less drastic in their effects on macromolecules than those which have been employed with such success in the classical studies of serological specificity.

Summary

Artificial desoxyribosenucleic acid-protein compounds were synthesized by introduction of a nitrobenzyl group into the nucleic acid, reduction to an aminobenzyl group, diazotization, and coupling to protein. Such compounds may prove useful in studies of nucleic acid structure and identity, especially if serological methods are to be employed.

References

1 Davidson, J. N.: *The biochemistry of the nucleic acids.* London, Methuen & Co., 1950.

2 Goebel, W. F. and Avery, O. T.: Chemo-immunological studies on conjugated carbohydrate proteins. IV. Synthesis of the p-aminobenzyl ether of the soluble specific substance of type III Pneumococcus and its coupling with protein. J. Exp. M., 1931, *54*, 431.

3 Gulland, J. M., Jordan, D. O., and Threlfall, C. J.: Desoxypentose nucleic acids. I. Preparation of the tetrasodium salt of the desoxypentose nucleic acid of calf thymus. J. Chem. Soc., Lond., 1947, p. 1129.

4 Haurowitz, F.: *Chemistry and biology of proteins.* New York, Academic Press. 1950.

5 Heidelberger, M. and Henriksen, S. D.: Proteic fractions of a strain of group A hemolytic streptococcus. IV. Azo dyes containing hemolytic streptococcal protein. J. Immun., Balt., 1941, *42*, 181.

6 Markham, R. and Smith, K. M.: Studies on the virus of turnip yellow mosaic. Parasitology, Lond., 1949, *39*, 330.

THE EFFECT OF COBALT-60 GAMMA RADIATION ON PASSIVE IMMUNITY*

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A considerable amount of work has been reported in the literature on the effect of x-rays on antibody formation and immunity. With the exception of the reports from our laboratory,[5, 11] the depressing effect of cobalt-60 gamma radiation upon immunity and antibody formation has not been reported. It would seem logical that x-rays and gamma rays should have a similar physiological effect. However, there is a greater difference between the wave length of the radioactive cobalt gamma ray and x-ray than there is between the antirachitic ultraviolet band and the ineffective band in the ultraviolet region. For this reason, we have chosen cobalt-60 gamma rays for our study of the effect of whole body ionizing radiation on the immunity mechanism in general.

Because of the excellent review of the effect of x-rays on immunity by Taliaferro and Taliaferro,[13] only references having a direct bearing on the experiments in this report will be included. Hollingsworth[7] has reported that whole body irradiation has no effect on the rate of destruction of passively administered antibodies. This was true for both homologous and heterologous serum.

Hale and Stoner[6] have also demonstrated that whole body gamma irradiation does not appreciably alter the disappearance of passively administered homologous tetanus antitoxin. Therefore, if sublethal whole body irradiation will depress the efficacy of such passively administered antibody, this procedure might be a means of studying the relationship of the cells of the passively immunized animal to the immunity produced by the antibody.

Experiments were designed to test the effect of whole body irradiation on passive immunity to pneumococcus Type III.

The radiation source was composed of twenty-one pieces of cobalt-60, arranged to give a uniform field at twelve inches from the source. Fifty mice could be irradiated at one time. The intensity at the time of these experiments was approximately 650 Rep. per hour and the LD_{50} for mice twenty-eight days of age‡ was 750 Rep. per hour.

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‡ The mice used in these experiments are the Swiss strain. They were raised in this laboratory under close supervision since 1938 and are free of the usual bacterial, viral, and parasitic diseases of mice.

Received for publication March 9, 1953.

A preliminary experiment indicated that the immunity-depressing effect increased markedly up to the third post-irradiation day. We did not try infecting later than the third post-irradiation day. From the work of Schechmeister, Bond, and Swift,[10] and Miller,[9] the immunity-depressing effect would be expected to increase up to about the thirteenth day after whole body irradiation.

The detrimental effect of cobalt-60 gamma irradiation on passive immunity to pneumococcus Type III is illustrated in Table 1.

TABLE 1

EFFECT OF COBALT-60 GAMMA RADIATION ON PASSIVE IMMUNITY IN MICE AGAINST PNEUMOCOCCUS TYPE III

Group	*No. of mice*	*Passively immunized with 0.3 ml. anti-pneumococcus rabbit serum*	*Radiation dosage*	*10^{-3} Pneumococcus broth culture injected intra-abdominally*	*Deaths*	*Percentage of deaths*
1	40	yes	650 Rep.	yes	36	90
2	40	yes	0	yes	0	0
3	40	no	0	yes	40	100
4	20	no	650 Rep.	no	0	0

TABLE 2

THE EFFECT OF COBALT-60 GAMMA RADIATION ON MOUSE RESISTANCE TO PNEUMOCOCCUS TYPE III MIXED WITH A 10-FOLD EXCESS OF SPECIFIC ANTISERUM. MICE WERE IRRADIATED THREE DAYS BEFORE THE INJECTION OF THE BACTERIAL AND ANTISERUM MIXTURE

Group	*No. of mice*	*Radiation dosage*	*Injected with a mixture of pneumococcus III & specific antiserum*	*Deaths*	*Percentage of deaths*
1	80	625 Rep.	yes	75	94
2	80	625 Rep.	no	0	0
3	80	0	yes	0	0

The mice in Group I were irradiated and passively immunized forty-eight hours later by intra-abdominal injection of 0.3 ml. of antipneumococcus rabbit serum. This was six times the amount required to protect against this amount of culture. The mice in Group II were also passively immunized at the same time. Seventy-two hours' post-irradiation, the animals in Groups I, II, and III were given 0.1 ml. of a 10^{-3} dilution of an eight-hour broth culture of Type III pneumococcus intra-abdominally. The mice in Group IV were irradiated but not infected, serving as controls on this amount of radiation. It is obvious that the 650 Rep. whole body irradiation effectively

destroys the protective power given to Group II by this amount of immune serum. The radiation controls in Group IV showed that no deaths were due to radiation alone over this period of time. The animals in Group III were not passively protected, and all were killed by the infection.

In view of these results, an experiment was designed to test the effect of gamma irradiation on the susceptibility of mice to pneumococci mixed *in vitro* with an excess of specific antiserum. From an examination of Table 2, it is clear that animals which received 625 Rep. three days before the injection of a mixture of pneumococcus Type III and specific antiserum were unable to withstand the infection. The organisms multiplied and killed 94% of the animals. No deaths occurred in Group II from radiation only. Also, there were no deaths in Group III which received only the mixture of pneumococcus serum and Type III organisms, showing that this mixture is harmless to non-irradiated mice.

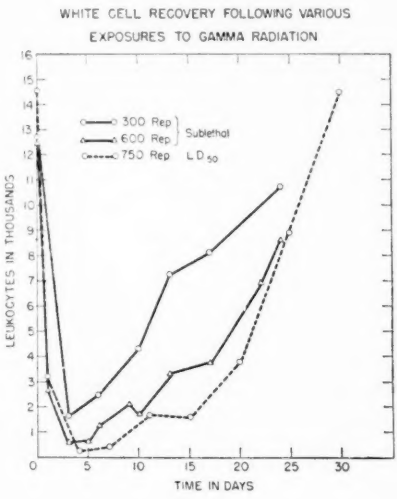

FIG. 1.

Although there are many reports stating that ionizing irradiation markedly depresses the leukocyte count, it seemed worthwhile to make total leukocyte counts following the amount of gamma irradiation used in these experiments on this particular strain of mice. The results of this study were plotted, and they are shown in Figure 1.

It seemed to us that the most logical explanation for the effect of irradiation on immunity to pneumococcus infection was the resulting marked reduction in white cells, and that it was possible that the resistance of irradiated and passively immunized mice might be restored by whole blood transfusion from normal mice or by the injection of a suspension of leukocytes from normal animals. An attempt was made to decrease the cobalt-60 gamma irradiation effects by injecting mouse leukocytes and whole citrated mouse blood.

The blood used was from adult mice bled from the heart under ether anesthesia, using a tuberculin syringe containing the appropriate amount of sodium citrate. The blood was pooled and immediately injected into the experimental mice.

TABLE 3

AN ATTEMPT TO DECREASE THE EFFECT OF COBALT-60 RADIATION ON IMMUNITY BY THE INJECTION OF MOUSE LEUKOCYTES AND WHOLE CITRATED MOUSE BLOOD

Group	*No. of mice*	*Passively immunized*	*Radiation dosage 3 days before infection*	*Whole blood given before infection*	*White blood cells given before infection*	*10^{-5} Pneumococcus 8-hour broth culture injected intra-abdominally*	*Deaths*	*Percentage deaths*
1	40	yes	650 Rep.	no	0.25 ml.i.v.	yes	36	90
2	40	yes	650 Rep.	0.4 ml.i.v.	no	yes	32	80
3	40	yes	650 Rep.	no	no	yes	34	85
4	40	yes	0	no	no	yes	0	0
5	40	no	0	no	no	yes	40	100
6	20	no	650 Rep.	no	no	no	0	0
7	20	yes	650 Rep.	no	0.25 ml.i.v.	no	0	0

The leukocyte preparation was obtained from young, adult mice. The donor mice were injected intra-abdominally with 4 ml. of sterile physiological saline solution. Four hours later these animals were sacrificed, the peritoneal fluid was removed aseptically, and a small amount of sodium citrate was added. This turbid fluid was rich in leukocytes. The suspension of cells was concentrated to one-fourth the original volume by centrifugation and immediately injected into the experimental animals. The results of this experiment are illustrated in Table 3.

The animals were irradiated three days before the challenge infection and those in Groups I, II, III, IV, and VII were passively immunized twenty-four hours prior to the challenge infection. The animals in Group I received leukocytes and those in Group II received citrated whole blood intravenously just before the injection of the pneumococcus Type III broth culture.

The results we have obtained show that neither the white blood cells nor the whole blood transfusions were effective in restoring the resistance enjoyed by non-irradiated animals in Group IV and that these animals were no better off than those in Group III. Group VI irradiated controls indicated no deaths due to radiation only. Group VII served merely as controls on the possibility of a bacterial contamination in the white blood cell suspension, indicating that the deaths were not due to such an accident.

We do not believe that this experiment should be considered conclusive by any means and we believe that better methods for obtaining leukocytes may eventually enable us to restore the resistance of such irradiated animals.

Similar experiments have been performed on passive immunity to tetanus toxin. The antitoxin was produced by active immunization of mice with purogenated tetanus toxoid.* Five-tenths ml. of antitoxic mouse serum was injected intravenously just before 625 Rep. gamma whole body irradiation. Non-irradiated mice of the same age and weight were also passively protected. The amount of antitoxin given was six international units. One thousand M.L.D. of tetanus toxin was given subcutaneously in the left flank seventy-two hours after the antitoxin injection and irradiation treatment. No paralysis occurred in either group of animals, indicating that the passive immunity was not effectively depressed.

It was thought that a more accurate quantitative test could be done if the toxin and antitoxic mouse serum were mixed *in vitro* and injected into irradiated and non-irradiated mice. Neutral and 90% neutral toxin were used to make the test as sensitive as possible. Although the mice used in this experiment were only five weeks of age, the necessity of observing them for only four days following the injection of toxin-antitoxin mixture permitted the amount of radiation used to be increased to 750 Rep. This is an LD_{50}

* We are indebted to Lederle Laboratories for a generous supply of this material.

for four-week-old mice. The results of this experiment may be observed by an examination of Table 4.

In spite of the intense radiation treatment, it is obvious that it made only a slight difference in the degree of paralysis between the irradiated and the control animals, while a great difference occurred in the similar experiment on pneumococcus Type III.

Although we doubt that the slightly greater degree of paralysis seen in the irradiated mice is of significance, it is in keeping with the observation of Adler and Schechmeister[1] which indicates that irradiated mice are slightly more susceptible to *Cl. septicum* toxin.

Although this report is primarily on the effect of gamma radiation on passive immunity, it may be of interest to include an experiment on the

TABLE 4

EFFECT OF COBALT-60 GAMMA RADIATION ON SUSCEPTIBILITY TO NEUTRALIZED AND UNDERNEUTRALIZED TETANUS TOXIN

Group	*No. of mice*	*Radiation dosage*	*Neutrality of the toxin-antitoxin mixture injected*	*Results*
1	20	750 Rep.	100%	Mild paralysis
2	20	0	100%	Just detectable paralysis
3	20	750 Rep.	90%	Moderate paralysis
4	20	0	90%	Mild paralysis

effect of cobalt-60 gamma radiation on active immunity to influenza Type A virus. The IA43 strain of influenza virus was used. This strain was isolated by one of us during the 1943 epidemic in Iowa.

The mice were actively immunized by two injections of 0.1 ml. of chorioallantoic fluid of chick embryos that had been infected with the virus forty-eight hours previously. The chicken red-cell hemagglutination titer was 1:1600. The interval between the two immunizing injections was twelve days. The first immunizing injection was subcutaneous and the second was intra-abdominal. The challenge infection was given fifteen days after the last immunizing injection. The virus used for immunization was not mouse-adapted. The challenge infection was by the same strain of Type A virus, which was adapted to mice. The challenge dose was an LD_{50} using titrated frozen virus. All mice were protected with an aqueous suspension of penicillin and dihydrostreptomycin mixture. Two thousand and 2,500 units, respectively, of each were injected subcutaneously just before the intranasal infection. These antibodies effectively prevent pneumonia in the irradiated mice from the nasal and oral flora which are washed into the lung by the intranasal inhalation. The influenza virus is not at all inhibited by the anti-

biotics. It is clear from an inspection of Table 5 that even 700 Rep. cobalt-60 gamma radiation had no effect in depressing the active immunity. The two deaths which occurred in Group I were undoubtedly due to the radiation since the autopsies did not show the usual plum-colored pneumonic lesion produced by influenza virus and the lungs were non-infectious when given to other mice. It is also clear that the mortality in the non-immune irradiated mice in Group III was no greater than it was in the non-irradiated, non-immune mice in Group IV.

DeGara and Furth[4] have reported that x-ray treatment of mice does increase their susceptibility. Our use of antibiotics may be responsible for this difference in results. They used influenza Type B, while we used Type A.

TABLE 5

EFFECT OF GAMMA RADIATION ON ACTIVE IMMUNITY TO INFLUENZA VIRUS

Group	*No. of mice*	*Immunized*	*Radiation dosage*	*Infected with type A Influenza virus*	*Deaths*	*Percentage of deaths*
1	36	yes	700 Rep.	yes	2	5.5
2	35	yes	0	yes	0	0
3	33	no	700 Rep.	yes	22	66.6
4	24	no	0	yes	16	66.5

Beulter and Gezon,[8] using a special method of infecting mice, found that with mouse-adapted virus there was no difference between irradiated and non-irradiated animals, but with chick-embryo-adapted virus there was a slight increased susceptibility.

The generally accepted view is that viruses are intracellular parasites and that propagation occurs only within cells. A coating of specific antibody on the surface of the virus particle may, and probably does, prevent the virus from making direct contact and entering the susceptible cell. Under these conditions, phagocytosis may not be necessary in antiviral immunity. This may explain why radiation is effective in destroying the immunity against the pneumococcus which can readily propagate outside the cells. This organism can propagate and kill experimental animals in the presence of abundant antibody if phagocytic cells are not present. This was shown by Pickrell[9] for immune animals intoxicated with alcohol and ether.

Generalization in biology is always hazardous. It would not be logical to assume that because the immunity to influenza virus cannot be broken by whole body gamma irradiation, that it would have no effect on natural species resistance to other virus disease. There is little, if any, evidence that natural species resistance to viral infection is related to the presence of specific antibody. It is entirely possible that the physiological alteration of

certain body cells by irradiation may well result in decreased natural resistance to some virus diseases, as has been demonstrated for Coxsackie virus in adult mice by Syverton.[12] Amoss, Taylor, and Witherbee[2] were able to increase the susceptibility of the monkey to poliomyelitis virus by large doses of x-rays, but they were unable to break the active immunity due to a previous infection.

So far as we are aware, there is no evidence that phagocytosis by leukocytes is necessary to prevent intoxication by neutralized tetanus toxin. Therefore, if whole body irradiation lowers resistance primarily because of the dramatic decrease in the leukocytic cells, one would not expect it to be effective in destroying active or passive immunity to tetanus toxin. We believe that these experiments indicate that this is the case for tetanus and for some viruses.

Conclusions

1. Cobalt-60 gamma whole body irradiation effectively destroys the immunity to pneumococcus infection even when abundant specific antibody is present.

2. Whole body irradiation does not cause an individual, having sufficient specific antitoxin present at the time of irradiation, to become susceptible to tetanus toxin.

3. The active immunity to influenza Type A virus is not altered by this form of irradiation.

References

1 Adler, F. L. and Schechmeister, I. L.: Effect of sublethal total body x-radiation on susceptibility of mice to *Clostridium septicum* toxin. Atomic Energy Commission—Unclassified, *2221*, 12.

2 Amoss, H. L., Taylor, H. D., and Witherbee, W.: Effects of large doses of x-rays on the susceptibility of the monkey to experimental poliomyelitis. J. Exp. M., 1919, *29*, 115.

3 Beulter, E. and Gezon, H. M.: The effect of total body x-radiation on the susceptibility of mice to influenza A virus infection. J. Immun., Balt., 1952, *68*, 227.

4 DeGara, P. F. and Furth, J.: The relative susceptibility of normal and x-rayed mice of different stocks to pneumotropic viruses. J. Immun., Balt., 1945, *50/5*, 255.

5 Hale, W. M. and Stoner, R. D.: The effect of cobalt-60 gamma radiation on antibody formation and immunity. International Record of Medicine, General Practice Clinics, 1952, *165/6*, 358.

6 Hale, W. M. and Stoner, R. D.: Unpublished work.

7 Hollingsworth, J. W.: Effects of x-irradiation on passively transferred antibody. Proc. Soc. Exp. Biol., N. Y., 1950, *75*, 477.

8 Miller, C. P.: Personal communication.

9 Pickrell, K. L.: The effect of alcohol intoxication and ether anesthesia on resistance to pneumococcal infection. Johns Hopkins Hosp. Bull., 1938, *53*, 238.

10 Schechmeister, I. L., Bond, V. P., and Swift, M. N.: The susceptibility of irradiated mice to infection as a function of post-irradiation time. Naval Radiological Defense Laboratory, Calif., 1950, AD-279(B).

11 Stoner, R. D. and Hale, W. M.: Effect of cobalt-60 gamma radiation on susceptibility and immunity to trichinosis. Proc. Soc. Exp. Biol., N. Y., 1952, *80*, 510.

12 Syverton, J. T.: Paper read before the American Society of Pathologists and Bacteriologists, April, 1952.

13 Taliaferro, W. H. and Taliaferro, L. G.: Effect of x-rays on immunity: A review. J. Immun., Balt., 1951, *66/2*, 181.

SOME COMMENTS ON GROWTH AND DEVELOPMENT IN ADOLESCENTS*

J. ROSWELL GALLAGHER† AND CONSTANCE D. GALLAGHER

"At first the little boy was short and fat and the little girl was long and thin, then the little girl became round and chubby while the little boy grew lanky and wiry. This was because the little girl used to sit very quiet and be good and the little boy used not."

James Stephen's description of these two young adolescents not only is couched in delightful prose but happily emphasizes the fact that adolescence is not a static but an ever-changing process. The reasons he assigns for the difference between the boy and girl may not be the true ones, but we should remember that it is only in recent years and because some of the more serious disorders of infancy and early childhood such as feeding problems, diarrheal diseases, and bacterial infections have become better understood, that physicians have begun to give their attention to the hitherto relatively neglected natural phenomena of adolescence.

These physicians, who are now both studying and caring for young people of this age group, find few *diseases* which are limited to adolescence, but they quickly discover that there are some matters concerning *these young people themselves* which should be kept in mind by the physician who advises and treats them. These matters (their concern with their own growth and development, their habit of strenuous living, their concern with schooling, and their emotional development) have been the subject of many publications, but a thorough understanding of one of them, the characteristics of the growth process in adolescence and the related fact of the adolescent's deep concern with his own growth, is so essential to a correct evaluation and proper management of a young boy or girl that reiteration is desirable.

Little children and adults have slight emotional concern with their size. An eight-year-old child does not care how tall or mature he is and a thirty-year-old adult does not become emotionally upset by the fact that his height is greater or less than his neighbor's. The adolescent, however, feels differently. Height, size, rate of growth, and state of maturity all mean a great deal during these years. The adolescent who is not maturing as rapidly as his or her companions, the boy who is shorter or the girl who is taller or more obese than usual, not only dislikes being different, but may become

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Received for publication March 5, 1953.

emotionally disturbed, fearing that she will keep on getting taller or that he or she will never mature. Some of this over-concern with their own growth and size is due to adolescents' conservatism, to their desire to be like their associates; some of it arises out of the fact that size is a factor in their athletic and social success; some of it develops because for years their parents, their teachers, and their physicians have expressed great interest in their growth and have compared them to charts and standards which children accept unquestionably and fail to understand. Few are aware that wide variation from the average is compatible with normality. Lastly, but very important as a source of anxiety, is their masturbation, in which they guiltily persist although fearing it may be retarding their growth.

Since adolescents feel as they do about growth, it is important to keep their attitudes in mind when dealing with them and to take time to correct their erroneous ideas when these are producing anxiety. They will need more than casual reassurance if their doubts regarding their normality are to be removed. Patient, clear explanation of the facts of growth and of individual variation may be no more effective at a single office visit than is an experienced algebra teacher's initial classroom effort. All this will probably need re-explanation, discussion, and reiteration, for not only is the physician faced with the usual factors which resist the absorption of new ideas, but he also has the handicaps of attempting to teach an anxious person and of trying to supplant long-standing misconceptions.

First, in dealing with adolescents, we need to remember that growth means more to these young people than it does to members of other age groups. Next, we need to remember to avoid reinforcing their already unwarranted beliefs regarding "standards" by ourselves avoiding careless or obsequious reference to them. Then we need patiently to explain the difference between deviating from the average and being abnormal; and to make clear the wide variation in time, rate, and extent of growth which exists among perfectly normal individuals. And lastly, but most important of all, as part of the patient's learning to understand and to accept himself, we need somehow to foster in these young people the idea of the uniqueness and the importance of the individual. None of this can be easily or quickly done, and to promote the idea of the importance of the individual in people whose world, while protesting a belief in freedom, bombards them with talk about, and the rewards of, average sizes, behavior, and attitudes, and unwittingly sets conformity on a pedestal, is as difficult as it is desirable.

Those studies of somatic growth which have involved the observation of the same adolescents over a period of several years have permitted generalizations which are of great value to the clinician. Data based upon the height or weight or state of maturity of members of a given group at a single point in time have some value, but information which is of much more valid application to an individual adolescent's growth problem is obtained from the year after year study of the growth patterns of the same boys and girls.

Out of such investigations one learns to have great respect for individual differences in rate and time and extent of growing. Even though all members of such a group are free from chronic illness or nutritional disturbances, they show wide variation in their mode of growth. If adolescents have anything in common, it would appear to be that each has his own individual growth pattern—that each differs from the others.

No concept is of more importance than this one of individuality in growth. There is a usual order in which the various characteristics of adolescence appear, but wide variation from this pattern is compatible with

TABLE 1

PREVALENCE OF EACH SKELETAL AGE AT EACH CHRONOLOGICAL AGE (BOYS)

Skeletal Age	*Chronological age*							
	13		*14*		*15*		*16*	
	Number	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*	*Number*	*Per cent*
12	4	6.7	5	2.3	1	0.5	0	0
13	22	36.7	21	9.8	8	3.7	1	0.9
14	25	41.6	87	40.7	37	17.3	3	2.7
15	7	11.7	66	30.8	57	26.6	20	17.7
16	2	3.3	31	14.5	76	35.4	32	28.3
17	0	0	4	1.9	26	12.2	38	33.6
18	0	0	0	0	9	4.2	19	16.8
Total	60		214		214		113	

normal growth. At age fourteen we find normal healthy boys and girls not only differing widely in skeletal and sexual development, in height and weight, but also in the time, rate, duration, and extent of their growth spurts. At age fourteen the *average* height and weight of a group of boys may be 66 inches and 127 pounds, but not only will many normal healthy boys vary all the way from 58 to 73 inches and from 75 to 190 pounds, but also there is good reason to believe that of the boys with identical chronological ages the largest will grow least and the smallest will grow the most during the next year. We have no evidence that within wide ranges any one time or any one way of growing is superior to another.

Since height and weight are the two measurements commonly used and because skeletal age cannot be ignored in adolescence, the relationship of these three to the concept of individual variation deserves the most detailed discussion. But other items, such as individual metabolic patterns or leg length or shoulder width or time of appearance of the various secondary sexual characteristics could be used to illustrate the same point. It is not just in these, but in all aspects of growth, that wide variation in adolescent

growth and development exists and to which *normal range* data brings much more valid information than do *averages.*

Skeletal Age and Maturation

When it is said that chronological age means little when applied to the adolescent years, reference is being made to the fact that at age fourteen for instance, healthy boys may vary all the way from a skeletal age (based on an evaluation of the hand-wrist X-ray) of from twelve to seventeen years, and all the way from one extreme of sexual maturity to the other. Table 1 shows the prevalence of each of the skeletal ages in one group of boys whose

TABLE 2

INITIAL AND SUBSEQUENT SKELETAL AGE AND MATURITY RATINGS OF FIVE BOYS WHOSE CHRONOLOGICAL AGES WERE FOUND TO BE MORE THAN TWELVE MONTHS GREATER THAN THEIR SKELETAL AGES

	Initial evaluation			*Evaluation One year later*			*Evaluation Two years later*		
Case	*C.A.*	*S.A.*	*Maturity group*	*C.A.*	*S.A.*	*Maturity group*	*C.A.*	*S.A.*	*Maturity group*
C.B.	14, 7	13, 6	3	15, 6	14, 5	4	16, 7	15, 3	4–5
D.C.	14, 9	13, 2	3	15, 8	14, 1	4	16, 9	15, 7	4–5
G.E.	15, 4	13, 9	3	16, 3	14, 6	4	17, 4	15, 9	4–5
H.S.	15, 11	14, 5	4	16, 11	15, 5	4–5	18, 0	16, 10	5
R.Z.	15, 7	13, 9	1	16, 7	15, 9	4–5	17, 7	16, 11	5

chronological ages ranged from thirteen to sixteen years. It will be seen that at chronological age fourteen these boys ranged all the way from skeletal age twelve to skeletal age seventeen. Only forty per cent of the group had similar chronological and skeletal ages.

It is important not only to realize that this variation in skeletal development exists, but also to know what subsequently happens to those who fall at the extremes. Here the advantages to be gained by serial observations of the same individual are emphasized. We rightly ask not what happens on the average, but what kind of growth pattern does each of these boys subsequently follow. Tables 2 and 3 provide an answer to that question.

Five boys in whom the skeletal age was more than twelve months below the chronological age at the time of the initial examination are represented in Table 2; and five boys whose skeletal ages were twenty-four or more months advanced appear in Table 3. Subsequent evaluations of skeletal age and maturity rating are shown in Table 2 and indicate that although these boys varied considerably in the way in which they approached skeletal maturity, they all were maturing, and, insofar as our present knowledge

permits us to judge, progressed in the manner most appropriate to each of them. These five boys continued to lag behind their chronological age standards, and they varied in the extent of their subsequent years' development, but all increased in skeletal age and in maturity rating. Only one gained more than twelve months in skeletal age rating during the first year, but four of the five gained more than twelve months in the second year and all but one gained twenty-four or more months in skeletal age over the two-year period. One fifteen-year-old boy (R.Z.), whose skeletal age was twenty-two months behind his chronological age at the time of his initial

TABLE 3

INITIAL AND SUBSEQUENT SKELETAL AGE AND MATURITY RATINGS OF FIVE BOYS WHOSE SKELETAL AGES WERE FOUND TO BE MORE THAN TWENTY-FOUR MONTHS GREATER THAN THEIR CHRONOLOGICAL AGES

	Initial evaluation			*Evaluation One year later*			*Evaluation Two years later*		
Case	*C.A.*	*S.A.*	*Maturity group*	*C.A.*	*S.A.*	*Maturity group*	*C.A.*	*S.A.*	*Maturity group*
J.D.	14, 5	16, 8	5	15, 5	16, 9	5	16, 6	18, 3	5
R.L.	14, 11	17, 3	4–5	15, 10	18, 3	5	16, 11	18, 9	5
A.M.	14, 6	16, 9	5	15, 5	17, 8	5	16, 6	18, 3	5
R.M.	15, 2	17, 3	5	16, 2	18, 0	5	17, 3	18, 9	5
J.P.	14, 9	17, 3	5	15, 9	18, 0	5	16, 10	18, 9	5

examination, gained thirty-eight months in skeletal age in the next twenty-four months and showed a striking change in his maturity rating.

The five boys in Table 3 whose skeletal ages each exceed their chronological ages by more than two years, though they continued to be considerably advanced in skeletal age, showed a trend toward a diminution in the difference between their skeletal and chronological ages. At the end of the two-year period, none had a two-year difference between his chronological and skeletal age.

Height

A prediction of future height at any given age needs to take into consideration family growth patterns. Adolescents vary not only in the extent, but also in the time at which they grow. Members of some families do most of their growing early in adolescence; others appear to stand still in their early teens and take a considerable spurt and continue to grow long after others of their same chronological age have ceased to increase in height. The *average* height at a given chronological age is of very limited value in the prediction of what an *individual's* growth will be: averages can only indicate *group* trends.

Table 4 which gives the *distribution* data upon which averages are based, however, is more helpful. It will be noticed that these averages are higher than those given in some other publications: this may be due to the fact that our data were collected from a group in which northern European stock predominated. The average heights based on skeletal age (Table 5) differ less from other published data than do our chronological data. That these data were obtained from the records of privileged and healthy boys

TABLE 4

DISTRIBUTION OF HEIGHTS AT CHRONOLOGICAL AGES 13-17 (BOYS)

	Age 13		*Age 14*		*Age 15*		*Age 16*		*Age 17*	
Height (inches)	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*
58	3	5.0	2	0.8	0	0	0	0	0	0
59	1	1.7	2	0.8	1	0.3	0	0	0	0
60	5	8.3	4	1.7	4	1.2	0	0	0	0
61	1	1.7	12	5.0	1	0.3	0	0	0	0
62	10	16.7	12	5.0	4	1.2	1	0.4	0	0
63	6	10.0	22	9.2	11	3.4	1	0.4	1	0.5
64	4	6.7	27	11.3	16	5.0	3	1.1	0	0.
65	13	21.6	27	11.3	27	8.4	14	5.0	5	2.6
66	5	8.3	28	11.7	34	10.9	30	10.7	14	7.3
67	4	6.7	28	11.7	50	15.6	31	11.1	27	14.1
68	4	6.7	30	12.6	46	14.3	41	14.6	29	15.1
69	2	3.3	29	12.1	47	14.6	52	18.5	32	16.7
70	2	3.3	4	1.7	37	11.5	46	16.4	31	16.2
71	0	0	5	2.1	22	6.9	21	7.5	18	9.4
72	0	0	5	2.1	9	2.8	20	7.2	15	7.8
73	0	0	2	0.8	10	3.1	12	4.3	7	3.6
74	0	0	0	0	1	0.3	6	2.1	7	3.6
75	0	0	0	0	1	0.3	2	0.7	6	3.1
Total	60		239		321		280		192	
Average		64.0		65.8		67.7		68.9		69.3

may render it more reassuring (when one consults it in regard to an individual boy who may be less privileged) than that obtained on a group more representative of the general population, for "if gold ruste, what shal iren do?"!

Table 4 makes it clear that normal young people vary tremendously in the extent to which they have grown at each age and that what is average for a group has little meaning for an individual. Ranges between which the heights of normal young people fall are obviously more significant than averages; to say that at age fourteen height varies from 58 to 73 inches in

normal healthy boys is more informative than to say that the average is 66 inches.

These wide ranges do not indicate that all efforts to predict future growth will be futile. If approached with an understanding of the limitation of what standard tables have to offer, individual guesses can be made. It is clear (Table 5) that guesses will be less subject to error if skeletal age standards rather than chronological age standards are used. That this is

TABLE 5

DISTRIBUTION OF HEIGHTS AT SKELETAL AGES 13-17 (BOYS)

	Age 13		*Age 14*		*Age 15*		*Age 16*		*Age 17*	
Height (inches)	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*
58	1	1.9	0	0	0	0	0	0	0	0
59	2	3.7	0	0	0	0	0	0	0	0
60	5	9.2	2	1.3	1	0.7	0	0	0	0
61	9	16.7	3	2.0	1	0.7	0	0	0	0
62	12	22.2	8	5.3	2	1.3	1	0.7	0	0
63	10	18.5	19	12.5	6	4.0	2	1.4	1	1.3
64	5	9.2	21	13.8	10	6.6	6	4.2	1	1.3
65	9	16.7	29	19.1	12	8.0	6	4.2	5	6.7
66	1	1.9	22	14.5	23	15.2	9	6.2	7	9.3
67	0	0	18	11.8	25	16.5	24	16.7	8	10.7
68	0	0	10	6.6	24	15.9	24	16.7	8	10.7
69	0	0	11	7.2	17	11.2	34	23.6	9	12.0
70	0	0	7	4.6	12	8.0	16	11.1	13	17.4
71	0	0	2	1.3	8	5.3	10	6.9	11	14.6
72	0	0	0	0	6	4.0	7	4.8	5	6.7
73	0	0	0	0	4	2.6	3	2.1	5	6.7
74	0	0	0	0	0	0	2	1.4	0	0
75	0	0	0	0	0	0	0	0	2	2.6
Total	54		152		151		144		75	
Average		62.4		65.5		67.4		68.4		69.1

true is suggested by the fact that there is no increase in average height after age fourteen in the chronological age-height table (Table 4), whereas there is an even yearly increase in height shown in the skeletal age-height table (Table 5). It is of interest that the chronological age-height averages of this group are greater than the skeletal age-height averages at each of the year periods except age seventeen when they become identical. This may be because this group had a high percentage of individuals whose skeletal age exceeded their chronological age (see Table 1). That the range of heights at each age below seventeen is wider in the chronological age table than in the skeletal age table is hardly surprising: both height and skeletal matura-

tion are affected by the same factors which are responsible for development during adolescene.

A height increment table, based upon chronological age (Table 6) indicates what we have become accustomed to accept as an unqualified fact, i.e. between the ages of thirteen and fifteen a greater height increment occurs in shorter than in taller boys. However the fallacy of that general-

TABLE 6

AVERAGE YEARLY INCREMENTS AND RANGES IN HEIGHT FOLLOWING CHRONOLOGICAL AGES 14, 15, AND 16 FOR VARIOUS HEIGHT LEVELS (BOYS)

	Age 14			*Age 15*			*Age 16*		
Initial height (inches)	*No. of cases*	*Average increment*	*Range*	*No. of cases*	*Average increment*	*Range*	*No. of cases*	*Average increment*	*Range*
58.0–58.9	1	4.0	4.0	...	..	.....	...	..	.....
59.0–59.9	2	2.4	1.8–3.0	...	..	.....	...	..	.....
60.0–60.9	3	2.7	2.0–3.0	3	3.0	2.0–3.5	...	..	.....
61.0–61.9	10	2.8	1.5–4.0	...	..	.....	...	..	.....
62.0–62.9	9	3.0	1.7–4.8	4	2.8	2.5–3.2	1	0.7	0.7
63.0–63.9	19	2.7	1.4–3.9	9	2.6	1.6–3.8	1	3.0	3.0
64.0–64.9	26	2.2	0.3–4.0	13	1.6	0.1–3.5	2	1.7	0.6–2.8
65.0–65.9	25	1.9	0.3–3.6	21	1.3	0.0–2.9	11	0.8	0.0–2.5
66.0–66.9	27	1.8	0.5–3.3	25	1.1	0.0–2.8	25	0.8	0.0–2.3
67.0–67.9	26	1.5	0.2–3.1	45	1.1	0.0–3.2	25	0.7	0.0–2.5
68.0–68.9	30	1.5	0.1–3.0	42	0.9	0.1–3.0	27	0.5	0.0–1.5
69.0–69.9	23	1.3	0.2–3.3	40	0.8	0.0–3.4	32	0.4	0.0–1.0
70.0–70.9	4	1.6	0.6–2.7	31	0.9	0.0–3.6	28	0.5	0.0–1.8
71.0–71.9	5	1.7	0.8–2.6	16	0.9	0.3–1.9	9	0.4	0.0–1.0
72.0–72.9	5	0.9	0.5–1.2	8	0.9	0.2–2.3	13	0.4	0.0–0.8
73.0–73.9	2	1.1	0.6–1.6	10	0.5	0.0–1.3	9	0.7	0.1–1.5
74.0–74.9	...	..	.....	1	0.0	0.0	4	0.3	0.0–0.5
75.0–75.9	...	..	.....	1	0.0	0.0	2	0.3	0.0–0.5
Total	217			269			189		
Average		1.9			1.1			0.6	

ization is apparent when one deals with an individual, and becomes particularly striking when that individual's skeletal age differs considerably from his chronological age. A real and practical difference exists between height increment data based on skeletal age and that based on chronological age (Table 6). From skeletal age-height increment tables (Table 7) we find that there is little difference in height increment at the same skeletal age whether the boy is short or tall. Two boys whose skeletal ages are fourteen but whose present heights are 70 inches and 62 inches respectively *both* may expect a height increase of about two to three inches in the next year.

Similarly at skeletal age sixteen, whether a boy is now short or tall, his expected height increment in the next twelve months is about the same.

What is likely to happen to a given boy whose present chronological age, skeletal age and height we know? Tables 6 and 7 suggest the answer. Greater growth occurs in the boys whose chronological and/or skeletal age is lowest, but there is little difference in the extent of subsequent growth in

TABLE 7

AVERAGE YEARLY INCREMENTS AND RANGES IN HEIGHT FOLLOWING SKELETAL AGES 14, 15, AND 16 FOR VARIOUS HEIGHT LEVELS (BOYS)

	Age 14			*Age 15*			*Age 16*		
Initial height (inches)	*No. of cases*	*Average increment*	*Range*	*No. of cases*	*Average increment*	*Range*	*No. of cases*	*Average increment*	*Range*
60.0–60.9	2	3.0	3.0	1	2.0	2.0	...	..	.....
61.0–61.9	3	2.7	1.5–3.3	...	..	.....	...	..	.....
62.0–62.9	5	2.9	1.8–3.3	1	1.7	1.7	1	0.7	0.7
63.0–63.9	18	2.9	1.8–5.3	3	1.6	1.4–1.7	2	1.6	1.5–1.6
64.0–64.9	18	2.7	1.4–4.0	8	1.6	0.3–2.8	6	0.9	0.8–1.2
65.0–65.9	22	2.5	0.5–3.6	10	1.2	0.5–1.7	6	0.9	0.3–2.5
66.0–66.9	21	2.3	0.8–3.3	21	1.4	0.5–2.7	7	0.7	0.2–1.1
67.0–67.9	17	2.4	1.5–3.2	24	1.4	0.7–2.0	23	0.6	0.0–1.7
68.0–68.9	8	2.2	0.3–3.0	22	1.2	0.9–2.0	22	0.7	0.0–1.5
69.0–69.9	11	2.4	1.3–3.4	12	1.1	0.2–1.9	33	0.7	0.0–1.5
70.0–70.9	7	2.4	1.8–3.6	9	1.5	0.6–2.2	11	0.6	0.0–1.2
71.0–71.9	2	2.3	2.0–2.6	5	1.3	0.8–1.9	8	0.8	0.5–1.0
72.0–72.9	...	..	.....	6	1.2	0.5–2.3	5	0.6	0.3–0.8
73.0–73.9	...	..	.....	4	1.5	1.3–1.6	3	0.8	0.5–0.9
74.0–74.9	...	..	.....	...	..	.....	2	0.2	0.0–0.4
Total	134			126			129		
Average		2.5			1.3			0.7	

boys of the same skeletal age whether they are at present short or tall. At the same skeletal age there is no significant difference in height increment between the shorter and the taller boys. There is, as one would expect, a gradual decrease in height increment as both chronological and skeletal age increase. This decrease seems to be independent of previous height if skeletal age is considered.

The increments based on chronological age, however, give a different picture. From the data in Table 6 it would appear that the future height increment at a given chronological age is dependent upon present height, that the shortest grow most, the tallest grow least. That one is less liable to error when one predicts height increments from skeletal age rather than from chronological age data is also indicated by Table 8. This table shows

the actual twelve-month height increments of seven fifteen-year-old boys whose skeletal ages differed from their chronological ages and whose heights ranged from 64 to 65 inches. These boys' skeletal ages ranged from thirteen to seventeen years. Their actual height increments and also the differences between the actual increments and the expected height increments (based on the data in Tables 6 and 7) are shown. In six instances the predictions based on skeletal age data were far superior to those based on chronological age data; in only one instance (E.B.) did the skeletal age data yield a much less accurate prediction than did the chronological age data.

TABLE 8

DIFFERENCES BETWEEN THE PREDICTED HEIGHT INCREMENTS BASED ON CHRONOLOGICAL AND SKELETAL AGE TABLES AND ACTUAL HEIGHT INCREMENTS IN SEVEN FIFTEEN-YEAR-OLD BOYS WHOSE SKELETAL AGES DIFFERED FROM THEIR CHRONOLOGICAL AGES AND WHOSE INITIAL HEIGHTS RANGED BETWEEN SIXTY-FOUR AND SIXTY-FIVE INCHES

Case	*Skeletal age*	*Actual Increment*	*Difference between actual and predicted increment (based on C.A. Table 4)*	*Difference between actual and predicted increment (based on S.A. Table 5)*
S.A.	14	2.5	+0.9	—0.2
J.B.	17	0.3	—1.3	0
L.D.	16	0.8	—0.8	—0.1
E.B.	14	1.4	—0.2	—1.3
D.D.	14	2.4	+0.8	—0.3
F.S.	16	1.2	—0.4	+0.3
J.A.	13	3.5	+1.9	+0.9
Standard error			1.041	.625

When one studies the subsequent growth of those boys who represented the ten per cent at each extreme of height for each skeletal age (Table 5) one finds that less than ten per cent of the short boys attained average height and that all of the tall boys remained above the average. However, the average yearly height increment of over sixty per cent of the short boys was greater than that for the skeletal age group as a whole, while fifty per cent of the tall boys subsequently grew less than the average yearly increment.

This concept of basing predictions upon developmental status rather than upon chronological age is of the utmost importance in the adolescent years. In these years of rapid and irregular growth, chronological age is frequently a misleading yardstick. Boys of fifteen or sixteen, worried about their stature and whose skeletal age is a year or two less than their chronological age can, providing no contradictory evidence exists, confidently be assured that further growth is likely.

Weight

During adolescence weight changes are considerable. There may be very striking increases in fat during early adolescence (which is too often confused with hypothyroidism), but usually increases in weight follow after rather than precede increases in height. A greater part of the weight increase in mid-adolescence is due to the increase in skeletal growth; in late

TABLE 9

DISTRIBUTION OF WEIGHTS AT CHRONOLOGICAL AGES 13-17 (BOYS)

	Age 13		*Age 14*		*Age 15*		*Age 16*		*Age 17*	
Weight (pounds)	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*
70-79	1	1.7	2	0.8	0	0	0	0	0	0
80-89	5	8.3	4	1.7	3	0.9	0	0	0	0
90-99	4	6.8	16	6.7	4	1.3	1	0.4	0	0
100-109	8	13.3	21	8.7	11	3.4	3	1.1	0	0
110-119	14	23.3	39	16.2	42	13.1	13	4.6	4	2.1
120-129	14	23.3	55	22.9	54	16.9	36	12.8	16	8.4
130-139	8	13.3	45	18.7	65	20.2	61	21.7	45	23.6
140-149	2	3.3	33	13.8	60	18.8	60	21.4	40	21.0
150-159	3	5.0	10	4.2	41	12.8	46	16.4	33	17.3
160-169	0	0	6	2.5	21	6.6	30	10.7	23	12.0
170-179	0	0	4	1.7	9	2.8	18	6.4	18	9.4
180-189	1	1.7	4	1.7	5	1.6	7	2.5	6	3.1
190-199	0	0	1	0.4	3	0.9	2	0.7	4	2.1
200-209	0	0	0	0	1	0.3	3	1.1	1	0.5
210-219	0	0	0	0	1	0.3	0	0	1	0.5
220-229	0	0	0	0	0	0	1	0.4	0	0
Total	60		240		320		281		191	
Average	118		127		138		146		150	

adolescence the increase is due chiefly to increase in the leg, thigh, and shoulder girdle musculature and partly to the deposition of fat.

Tables 9 and 10 list the ranges of weight; and Tables 11 and 12 show the yearly increments in weight of adolescent boys. These tables show higher weights than do those of other observers; this is undoubtedly due to the factors already mentioned. The skeletal age-weight tables seem to offer little, if any, advantage over the chronological age-weight tables, but the extraordinary range in weights and in yearly weight increments (Tables 11 and 12) emphasizes the value of distribution data and their superiority to tables which give only averages.

What can one expect will happen to those boys who fall at the extremes of weight but who are in good health emotionally and physically and require

no treatment? All those boys who represent the ten per cent at the lower extreme of weight for each skeletal age group at their initial examination remained below average weight throughout the period of observation. Only one-third of this group had, at any time, a weight increment greater than average. Similarly, all those boys who were in the ten per cent group, comprised of the heaviest boys, remained above average weight. Fewer than half of these boys showed less than the weight increment which was average for their age.

TABLE 10

DISTRIBUTION OF WEIGHTS AT SKELETAL AGES 13-17 (BOYS)

	Age 13		*Age 14*		*Age 15*		*Age 16*		*Age 17*	
Weight (pounds)	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*	*No. of cases*	*Per cent*
80-89	4	7.4	0	0	0	0	0	0	0	0
90-99	12	22.2	10	6.7	0	0	0	0	0	0
100-109	16	29.6	16	10.5	5	3.3	0	0	0	0
110-119	13	24.1	36	23.6	20	13.6	9	6.3	3	4.0
120-129	6	11.1	43	28.2	34	22.6	23	16.1	11	14.7
130-139	1	1.9	28	18.4	32	21.3	30	21.0	12	16.0
140-149	0	0	10	6.7	33	22.0	36	25.2	20	26.7
150-159	2	3.7	5	3.3	11	7.3	25	17.5	11	14.7
160-169	0	0	1	0.7	10	6.7	7	4.9	10	13.3
170-179	0	0	0	0	2	1.3	8	5.6	6	8.0
180-189	0	0	3	2.0	2	1.3	3	2.1	0	0
190-199	0	0	0	0	0	0	1	0.7	1	1.3
200-209	0	0	0	0	0	0	1	0.7	0	0
210-219	0	0	0	0	1	0.7	0	0	0	0
220-229	0	0	0	0	0	0	0	0	1	1.3
Total	54		152		150		143		75	
Average	108		124		136		145		147	

Factors such as dietary habits, emotional disorders, illness, and constitutional differences are at times a satisfactory explanation of the failure of some lean individuals to gain and of some other overweight adolescents to lose weight, but in certain instances no apparent cause is found. Regardless of their determined efforts to gain or lose, some adolescents meet with little success in modifying their weight. Since his group's early studies may point the way toward an explanation of this puzzling problem and because they show that individuals vary in their metabolic patterns even as they do in other respects, Williams' work should be kept in mind. His data suggest that these obese individuals have characteristic metabolic differences. Further studies may run counter to this preliminary investigation, but the

TABLE 11

AVERAGE YEARLY INCREMENTS AND RANGES IN WEIGHT FOLLOWING CHRONOLOGICAL AGES 14, 15, AND 16 FOR VARIOUS WEIGHT LEVELS (BOYS)

	Age 14			*Age 15*			*Age 16*		
Initial weight (pounds)	*No. of cases*	*Average increment*	*Range*	*No. of cases*	*Average increment*	*Range*	*No. of cases*	*Average increment*	*Range*
70-79	2	12.5	11-14	...	...	.....	...	..	.....
80-89	2	16.5	16-17	2	15.0	10-20	...	..	.....
90-99	15	14.6	8-26	2	12.5	10-15	...	..	.....
100-109	17	14.1	6-21	7	10.3	7-13	2	9.5	7-12
110-119	34	12.3	1-24	35	11.8	1-28	9	4.1	(-3)-10
120-129	48	9.6	(-6)-20	47	9.3	3-43	31	5.6	(-4)-14
130-139	45	11.4	(-6)-26	57	7.7	(-6)-33	44	5.6	(-3)-18
140-149	31	11.2	(-2)-40	51	7.0	(-7)-27	37	4.3	(-11)-14
150-159	8	10.4	(-5)-32	32	7.3	1-19	27	5.6	(-6)-14
160-169	5	8.6	0-14	19	7.5	(-12)-37	19	2.6	(-25)-16
170-179	4	9.8	(-7)-18	8	8.0	2-19	12	1.6	(-16)-12
180-189	3	16.0	(-1)-25	5	2.0	(-12)-18	4	1.0	(-11)-9
190-199	1	(-9.0)	(-9)	2	18.0	6-30	2	5.0	4-6
200-209	...	....	.....	...	...	.....	3	(-2.7)	(-22)-13
Total	215			267			190		
Average		11.4			8.5			4.5	

TABLE 12

AVERAGE YEARLY INCREMENTS AND RANGES IN WEIGHT FOLLOWING SKELETAL AGES 14, 15, AND 16 FOR VARIOUS WEIGHT LEVELS (BOYS)

	Age 14			*Age 15*			*Age 16*		
Initial weight (pounds)	*No. of cases*	*Average increment*	*Range*	*No. of cases*	*Average increment*	*Range*	*No. of cases*	*Average increment*	*Range*
90-99	9	12.4	8-17	...	...	....	...	...	....
100-109	10	14.2	6-19	4	9.0	3-13	...	...	....
110-119	32	14.2	4-24	16	9.0	1-14	8	7.0	3-12
120-129	40	11.9	(-2)-24	29	9.6	(-6)-43	19	6.3	1-19
130-139	27	15.4	4-22	30	9.7	2-19	28	6.8	(-2)-26
140-149	9	17.1	11-40	29	8.9	(-7)-22	35	7.7	(-2)-27
150-159	4	23.5	17-28	9	6.6	(-5)-18	20	7.2	2-19
160-169	1	26.0	26	7	6.9	(-12)-12	7	5.9	(-25)-37
170-179	...	...	.....	2	14.0	11-17	6	7.8	0-19
180-189	3	17.3	9-25	1	24.0	24	3	4.3	(-1)-9
190-199	...	...	.....	...	...	.....	1	30.0	30
Total	135			127			127		
Average		14.3			9.2			7.2	

approach seems promising, and the general concept that every individual has a characteristic biochemical pattern which is distinctive for him alone and most similar to that of those others who resemble him most closely has fascinating implications. It has everything to do with individual differences and nothing in common with studies which emphasize the average measurements of a variety of phenomena in a mythical average man.

Progress in growth is more important than is an adolescent's present status. That he is gaining height or is moving toward adulthood in any way is the significant fact. Changes in skeletal development, in total height, in leg length, in trunk height, in hip and shoulder width, all occur in adolescence but at varying ages and with variation in pattern. Adolescence is not static—it is a process; the Romans packed *motion* into their verbs which end in *"scens."* That change is occurring in some one of these aspects of growth suggests that further and more usual changes in other respects will shortly appear. It is when *no change* is taking place and when the skeletal age is far advanced beyond the time for such changes that one should suspect a pathological condition. In boys, usually one first sees a gradual increase in size of genitalia; then the appearance and development of pubic, then facial, then axillary hair. In girls, one first notices a budding of the breasts, next an appearance of pubic hair, and then the menarche. The timing, their sequence, and the extent of these vary among different individuals. They are important straws in the wind, however, and when, for instance, a boy whose present height is at the lower limits of normal shows evidence of growth in his leg length, or, when seeming immature, pubic hair begins to develop, these are reassuring signs and indicate that the growth phenomena of adolescence are beginning to emerge.

These comments and these data illustrate the fact that normal, healthy individuals show wide differences in various growth phenomena, that the vast majority of them reach a satisfactory and what may well be for each of them an ideal adult state though their way of reaching that goal varies considerably. Though these are facts, and though the individual would do well, except in rare instances, to accept himself as he is and not wish to imitate some standard pattern, his physician should recognize the psychological potentialities of wide differences from the usual, and anticipate those anxieties which are so likely to develop in adolescents who find themselves to be very different. It is one thing to understand that these differences frequently occur and are within normal limits, and quite another thing to ignore them and to forget that they can be the source of real anxiety and of a variety of psychosomatic disorders in an adolescent. In dealing with adolescents, therefore, it is necessary to appreciate how much they dislike being different and how anxious they may become when they believe they are abnormal, and to have constantly in our own minds not average data and standards but instead a clear picture of the wide variations which are

still well within normal limits. We adults, unwittingly perhaps, seem to prefer that others conform to the average. Too often there is the implication that thinness and fatness are, *ipso facto,* undesirable. Admittedly, to weigh more or less than he does, or to be taller or shorter than he or she is, might in general be desirable for many adolescents, but are we always sure for this particular adolescent, *with his constitution,* that what is nearer the average is necessarily *optimal for him?* We all know many deviates who, apparently having accepted themselves as they are, and not perennially (and futilely) seeking to be otherwise, lead happy, healthy, and useful lives.

Judge Learned Hand's warning would seem to have medical as well as political implications and to be peculiarly apropos: "Our dangers, as it seems to me," he said, "are not from the outrageous but from the conforming; not from those who rarely and under the lurid glare of obloquy upset our moral complaisance or shock us from unaccustomed conduct, but from those, the mass of us, who take their virtues and their tastes, like their shirts and their furniture, from the limited patterns which the market offers. . . . Since our ancestors fully straightened their knees and rose upon their hind legs to become Homo Sapiens, there have never been one-tenth as many people in the world who felt alike, thought alike, ate alike, slept alike, hated alike—so far as they hate at all—loved alike, wore the same clothes, used the same furniture in the same houses, went to the same games, saw the same plays, approved the same sentiments, believed in the same God, and, most important of all, were all confidently assured that nothing was lacking to their complete realization of the Human Ideal. Over that chorus the small voice of the individual sounds not even the thinnest obbligato; it seems senseless and preposterous to sing at all. Why not accept the accredited chant and swell the din?"

References

Frank, Lawrence: Chapter 91, Growth, development and physiological functioning of adolescents, in: *The child in health and disease,* C. G. Grulee and R. Cannon Eley, Eds. Baltimore, Williams and Wilkins Co., 1952.

Gallagher, J. Roswell: *Understanding your son's adolescence.* Boston, Atlantic-Little, Brown & Co., 1951. 212 pp.

Greulich, W. W. and Pyle, I. S.: *A radiographic atlas of skeletal development of the hand and wrist.* Stanford, Stanford University Press, 1950. 190 pp.

Hand, Learned: *The spirit of liberty.* Papers and addresses of Learned Hand (collected by Irving Dilliard). New York, A. A. Knopf, 1952. 262 pp.

Stephens, James: *The crock of gold.* New York, The Macmillan Co., 1913. 298 pp.

Stuart, Harold C.: *Normal growth and development during adolescence.* N. England J. M., 1938, *234,* 666-672, 693-700, 732-738.

Williams, R. A., *et al.: Biochemical Institute Studies IV.* Austin, University of Texas Publications, Number 5109, 1951.

Acknowledgment

The authors wish to express their appreciation to the publishers of the papers of Judge Learned Hand and of James Stephens' *Crock of Gold* for permission to quote from these books.

GROUP A POLYSACCHARIDE PRECIPITIN REACTIONS IN ACUTE STREPTOCOCCOSIS AND RHEUMATIC FEVER*

LOUIS WEINSTEIN†

The rôle of the beta-hemolytic *Streptococcus* in the etiology of rheumatic fever has attracted considerable attention for the last twenty or more years. Although epidemiological studies, particularly those indicating a high incidence of streptococcic pharyngitis preceding first attacks or recurrences of rheumatic disease, have furnished highly suggestive evidence of the importance of this organism in the pathogenesis of this disorder, the exact mechanisms involved have not yet been elucidated. The development of a hypersensitivity reaction to *Streptococcus pyogenes* or to some of its endo- or extracellular products has been suggested by many investigators mainly on the basis of two observations: (i) the presence of a "latent period" between the initial streptococcal infection and the subsequent development of rheumatic fever, and (ii) the appearance of various antibodies to the *Streptococcus* and its products with greater frequency, in higher concentration, and persisting for a longer time in the rheumatic state than in uncomplicated streptococcosis.

A large number of studies of specific and non-specific serologic reactions have been carried out in an attempt to characterize the immunological reactive pattern of the rheumatic and to contrast it with uncomplicated streptococcal infection in order to detect differences that might be of help in unmasking the mechanisms involved or in establishing the early diagnosis of the rheumatic state. Investigations of the serum content of anti-streptolysin, anti-streptokinase, anti-M, anti-C, anti-hyaluronidase, anti-X, antitrypsin, C-reactive protein, beta-hemolytic streptococcal agglutinin and bactericidal antibody, among others, have been thought to indicate that some of these appear more frequently, are present in larger quantities, and persist longer in patients with rheumatic fever than in those who have uncomplicated streptococcosis. None of these studies, however, have succeeded in attaching an unquestionable direct causal relationship of any of these immune substances to the pathogenesis of rheumatic fever. Careful evaluation of the voluminous literature on this subject, which cannot be reviewed in this paper because of limitations of space, permits only one conclusion—positive reactions with the various antibodies mentioned above

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Received for publication March 13, 1953.

indicate no more than recent experience with the beta-hemolytic *Streptococcus*. Although it has been suggested that their presence in larger numbers of patients, their higher titers, and longer persistence can be explained on a greater degree of immunological reactivity of the rheumatic subject, this still remains to be proved.

The purpose of this paper is to report the results of a search for antibody to one of the endocellular materials of the *Streptococcus*, the group specific carbohydrate or C substance, in the serums of patients with scarlet fever, rheumatic fever, and a number of unrelated infections in order to determine its possible importance in the pathogenesis of rheumatic fever, and to evaluate its significance in the diagnosis of this disease.

Most of the investigations of the incidence and significance of antibody to specific streptococcal polysaccharide have been carried out in cases of rheumatoid and other types of arthritis. Using crude preparations of this carbohydrate, Dawson and his co-workers[4] reported a much higher incidence of positive reactions with the serums of rheumatoid arthritics than with those of individuals who had scarlet fever, glomerular nephritis, osteoarthritis, hypertension, diabetes mellitus, bacteremia due to a non-hemolytic *Streptococcus*, and "non-articular rheumatism." Bruce and Caswell[1] employed crude hydrochloric acid extracts of the beta-hemolytic *Streptococcus* and found that 68.7 per cent of a group who suffered from rheumatoid arthritis had anti-C antibody in their blood, while 13 patients with osteomyelitis and 10 normal people did not. A more extensive investigation of this problem was carried out by McEwen, Bunim, and Alexander[8] who used crude extracts made from the N Y 5 strain of *Strep. pyogenes*. The percentage of positive precipitin reactions noted in various diseases was as follows: Normal people—24; hemolytic streptococcal disease—62; active rheumatic fever with carditis and chorea—55; rheumatic fever with polyarthritis—56; active rheumatoid arthritis—77; gonococcal arthritis—32; non-specific, low grade, pyogenic arthritis—31; osteoarthritis—18; gout and tuberculous spondylitis—12; unclassified, infectious arthritis—46; and wholly unclassified arthritis—58. Although the incidence of precipitating antibody for crude extracts of the beta-hemolytic *Streptococcus* was somewhat higher in patients with rheumatoid arthritis than in those who did not have this disease, the differences do not appear to be of significant proportion. About half of a group of rheumatoid arthritics was found by Neil and Hartung[9] to have antibody for a combination of M and C substances, while normal individuals or those with osteoarthritis rarely or never exhibited this precipitin.

Two important studies of the presence of antibody to various of the group specific carbohydrates of *Strep. pyogenes* are those of Dawson and Olmstead[5] and Chassis and McEwen.[2] These investigators carried out a search for precipitins to the polysaccharides of groups A, B, C, D, E, F, and G *Streptococcus*. They found that a very high percentage of patients

with rheumatoid arthritis gave positive reactions with group A extracts, but that the number decreased with the other carbohydrates studied, being lowest with E, F, and G. Precipitation was observed with normal sera only occasionally when the A substance was used.

Reports of the presence of precipitin for specific streptococcal carbohydrate in rheumatic fever and acute streptococcal pharyngitis are few and controversial. This antibody could not be detected by Coburn and Pauli[9] in a large number of individuals with rheumatic fever. Seegal and his coworkers[11] found the highest incidence of positive anti-C reactions in cases of rheumatoid arthritis but demonstrated them also in about 45 per cent of patients with acute hemolytic streptococcal infections, in one-third of those with endocarditis due to *Strep. viridans,* and in 12 per cent of acute glomerular nephritides. Studies of normal controls and of people with noninfectious diseases revealed this precipitin in from zero to 10 per cent. In a small group of cases of rheumatic fever, Chassis and McEwen[8] detected positive reactions with group A polysaccharide in 100 per cent; in 8 patients with scarlet fever only 25 per cent exhibited this antibody. The most recent study of this subject is that of Rantz and Randall[10] who reported that about 8 per cent of people suffering Group A hemolytic streptococcal infections developed precipitating antibody for the specific polysaccharide during the course of their illness. The immune response was usually maximal by the end of the fourth week; no relationship could be established between it and "the nature of the initial illness" or the development of suppurative complications. In patients in whom arthritis appeared late in the course of the disease anti-C precipitins were demonstrable frequently, either initially or during convalescence, but in those with late fever, they never appeared. When carditis supervened late in the course of the disease, about 6 per cent of those who had no detectable antibody at the start of their illness developed it during recovery.

The studies reported in this paper indicate that precipitating antibody for the group-specific carbohydrate of Group A *Streptococcus pyogenes* is detectable with no greater frequency in patients with rheumatic fever than in those who have uncomplicated streptococcal pharyngitis. As a matter of fact, this precipitin is present so often in many unrelated infections that it appears to have little or no significance even in detecting recent infection with the beta-hemolytic *Streptococcus.*

Materials and Methods

Five hundred and thirty-seven patients admitted to the Haynes Memorial Hospital for the treatment of a large variety of infectious diseases were the subjects of this study. Cultures of the nose and throat were made in every case on the day of admission and on the following day. All of the streptococci isolated were subjected to grouping and typing. Ten milliliters of blood were withdrawn aseptically from each person at the time of admission and again on the fourteenth day, wherever possible. In those with known streptococcal infections, blood was obtained on the day of entry to the

hospital, 14 days later, and at the time of discharge. After clotting, the serum was removed and stored in the deep freeze refrigerator until tested.

The group specific polysaccharide was prepared in the following manner. A strain of *Streptococcus pyogenes*, Group A Type 3, which had been frequently passed into mice in order to maintain a high degree of virulence, was the source of the carbohydrate used in this study. The bacteria from 30 liters of heart-infusion-yeast-tryptose broth were obtained by centrifugation after 18 hours of incubation at 37° C. They were suspended in 200 ml. of N/20 HCl and heated for 10 minutes in a boiling water bath. Extraction in hydrochloric acid was repeated six times and the extracts pooled. After clearing by centrifugation, the combined extracts were neutralized with N1 NaOH. The precipitate which formed was discarded and to the supernatant were added 20 gms. of sodium acetate per liter and 3-4 volumes of 95 per cent ethyl alcohol. Most of the type-specific nucleoprotein (M substance) has been shown to be precipitated by this procedure and the polysaccharide to be left in solution, according to Lancefield.[6] The supernatant fluid was concentrated to a small volume *in vacuo* at a temperature not exceeding 37° C. and the salts dissolved in distilled water. Three volumes of alcohol were added, the solution placed at ice box temperature overnight and the precipitate then removed by centrifugation. The supernatant was again concentrated to a small volume *in vacuo* at a temperature not above 37° C. This material was dialyzed through a parchment bag because Lancefield had found that the group specific polysaccharide was dialyzable through parchment but not through special collodion membranes. Advantage of this phenomenon was taken in order to shorten the process of purification previously described by Lancefield.[7] Dialysis was set up in a closed system against distilled water and allowed to proceed for twenty-four hours in the ice box. The dialysate was removed and the procedure repeated five times. All of the dialysates were then pooled and three volumes of ethyl alcohol added. A very small quantity of precipitate resulted. After centrifugation, the supernatant was again concentrated *in vacuo* to dryness. The material was dissolved in 0.85 per cent NaCl solution. Examination for the presence of Type 3 M substance and Group A polysaccharide, using potent specific antisera, revealed complete absence of the type specific nucleoprotein and large quantities of carbohydrate A. This solution, diluted to the point where it gave 4 plus reactions with potent anti-Group A serum after 2 hours of incubation at 37° C. and 24 hours' storage in the ice box, was the one used in performing all of the precipitin tests reported in this paper. All of the tests were set up in duplicate and all, whether positive or negative, were repeated twice.

The following method was used to detect the presence of precipitating antibody for the purified Group A carbohydrate. The C-containing solution was layered over the patient's serum in a capillary precipitin tube and incubated at 37° C. for two hours. After 24 hours' storage in the ice box all the tubes were examined for precipitation and again placed at low temperature. Readings were made again after 48 and 72 hours of ice box storage. For every test, a control consisting of the bacterial extract plus saline and of the patient's serum plus saline was set up. The results were not recorded in terms of degree of precipitation but only as positive or negative.

Results

Streptococcal Infections

One hundred twenty patients with scarlet fever were studied. The serum of 40 (33.3 per cent) showed precipitation when mixed with the purified group-specific polysaccharide. Seventy-five per cent of these had C-precipitin at the time of admission to the hospital but in the remainder,

this antibody was absent in the initially drawn serum but was present 14 days later. There appeared to be no correlation between age of the patients, the severity of the disease, the persistence of streptococci in the pharynx, and the development of suppurative or non-suppurative complications, and the appearance of anti-polysaccharide precipitins.

Seven cases of erysipelas were examined; none of them developed antibody for the C substance. Of 24 individuals with suppurative otitis media due to *Streptococcus pyogenes,* 12 (50 per cent) exhibited the presence of carbohydrate precipitin two weeks after admission to the hospital. In none of them was there any relation between severity and complications of the primary disease and the development of this immune reaction.

Seventy-six patients with acute rheumatic fever were studied. All of them had obvious clinical rheumatic disease; those with questionable syndromes were excluded from the investigation. The precipitin reaction was positive in only 18 (23.7 per cent). In every instance in which this antibody was detected it was present at the time of admission to the hospital. None of those which were initially negative subsequently became positive. There was no relation between the presence or degree of severity of carditis or arthritis or the duration of rheumatic activity and the development of polysaccharide precipitin.

Only six cases of acute glomerulonephritis were available for study. Of these, three (50 per cent) gave a positive reaction for streptococcal group specific polysaccharide at the time of admission to the hospital; in the others, this antibody was not detectable even late in the course of the disease.

The results obtained indicate that the development of precipitating antibody for purified group A specific polysaccharide of the beta-hemolytic *Streptococcus* does not take place in every patient infected by this organism. There also does not seem to be any relation between severity of the primary streptococcal disease or of its complications and the frequency with which this type of immune reaction appears. It is quite striking that in erysipelas this precipitin was never detected; this observation must be qualified, however, by the fact that only a few patients with this disease were studied. Most interesting is the demonstration of a somewhat lower incidence of positive reactions in patients with frank rheumatic fever than in those who had streptococcic pharyngitis without developing this complication. Although the frequency of positive reactions in acute glomerulonephritis was somewhat higher than in scarlet or rheumatic fever, this may be accounted for by the relatively small number of cases examined.

These data suggest that the development of antibody to the group-specific carbohydrate of the *Streptococcus* is without significance either in the diagnosis of Streptococcosis (this point is further emphasized by the results presented below) or in the pathogenesis of rheumatic fever or glomerulonephritis. Absence of this precipitin does not rule out the presence of

streptococcic infection, since at least two-thirds of the patients with scarlet fever never developed it even during the course of persistent streptococcic infection despite the fact that some of them exhibited suppurative complications due to *Strep. pyogenes.*

Infections Other Than Those Due to the Streptococcus

In order to evaluate the importance of the presence of precipitin for group A polysaccharide in the blood of patients with infections due to *Streptococcus pyogenes,* the serum of 304 individuals with illnesses due to

TABLE 1

INCIDENCE OF PRECIPITIN REACTIONS WITH GROUP A POLYSACCHARIDE IN VARIOUS STREPTOCOCCAL AND OTHER DISEASES

Disease	*No. of patients*	*No. of positive precipitin reactions*	*Per cent positive precipitin reactions*
Scarlet fever	120	40	33.3
Strep. otitis media	24	12	50.
Erysipelas	7	0	0.
Acute rheumatic fever	76	18	23.7
Glomerulonephritis	6	3	50.
Diphtheria carriers	3	3	100.
Acute diphtheria	42	5	11.9
Scabies	61	25	41.
Syphilis	42	7	16.7
Rubella	3	1	33.3
Varicella	20	3	15.
25 other diseases	133	0	0.

other bacteria, viruses, and other parasites were examined. The diseases studied and the number of patients (in parentheses) in each group were as follows: *Bacterial infections*—pertussis (17), pulmonary tuberculosis (15), diphtheria (42 active cases and 3 asymptomatic carriers); meningitis (5), suppurative otitis media (3), and tracheitis (2) due to *H. influenzae:* meningitis due to *Ps. aeruginosa* (3), meningococci meningitis (2), gonorrhoea (2), staphylococcal osteomyelitis and bacteremia (1), tracheitis due to *Strep. viridans* (1), bronchopneumonia due to various organisms including *D. pneumoniae* (5), and fusospirochetal angina (2). *Virus infections*—measles (28), varicella (20), mumps (7), infectious mononucleosis (6), rubella (3), viral diarrhea of infants (2), probable viral pharyngitis (19), viral tracheitis (1), and infectious hepatitis (1). *Miscellaneous infections and other diseases*—scabies (61), secondary and tertiary syphilis (42), monilial stomatitis (2), cervical adenopathy of unknown etiology (2), dermatitis (3), appendicitis (1), and scurvy (1).

Precipitin for group A specific carbohydrate of the beta-hemolytic *Streptococcus* was demonstrable only in the following diseases: diphtheria, varicella, rubella, scabies, syphilis, and in the asymptomatic diphtheria carrier state. The antibody was present most frequently in diphtheria carriers—100 per cent; the significance of this is difficult to evaluate, however, since only three cases were investigated. In 61 individuals with scabies, 25 or 41 per cent gave positive reactions. Fifteen per cent of the instances of varicella, 33.3 per cent of those with rubella, 14.9 per cent of the syphilitics, and 12.5 per cent of the patients with active diphtheritic pharyngitis yielded precipitins for the C substance. The remaining 133 persons suffered from 25 different disease processes, mostly various infections. Specific streptococcal carbohydrate precipitin was not detected in any members of this group. The results of the entire study are presented in Table 1.

Discussion

Many of the investigations that have been carried out in an attempt to demonstrate precipitating antibodies for the group specific polysaccharide of Group A beta-hemolytic *Streptococcus* have been somewhat confused by the use of crude preparations which contained not only the carbohydrate, but also type specific nucleoprotein M and the non-specific nucleoprotein P. The data presented in this paper were obtained with highly purified streptococcal polysaccharide. Advantage was taken of Lancefield's observation[7] that the group specific substance is dialyzable through ordinary parchment membranes. The method of purifying the material which was used in the present study was thus made more simple while the degree of purity was increased at the same time. As was pointed out above, the preparation employed was entirely free of the type-specific nucleoprotein as shown by its failure to be precipitated by potent Type 3 specific antiserum.

The results obtained in various types of streptococcal disease are somewhat at variance with other published reports. Unlike the studies of Coburn and Pauli[8] who could not demonstrate precipitin for streptococcal carbohydrate in the serums of patients with pharyngitis or rheumatic fever, or those of Chassis and McEwen[9] who found this antibody in 100 per cent of rheumatics, the present investigation revealed that about one-third of cases of scarlet fever and about one-fourth of those with rheumatic fever had positive reactions. The data of Rantz and Randall[10] suggest a lower incidence of anti-C antibody—8 per cent in Group A hemolytic streptococcal infections. The failure to detect the C-precipitin in seven cases of erysipelas cannot be explained. The observations demonstrating a lack of relationship between the severity of the initial disease or the appearance of suppurative complications and the development of the immune reaction are in accord with those of Rantz and Randall.[10]

Most striking is the fact that not all of the patients with proved streptococcal infections exhibited precipitating antibody for the group-specific

carbohydrate. This may be due to the relatively poor antigenicity of the polysaccharide even in its natural state in the organism. It is well known that injection of whole streptococci into experimental animals leads to the development of active C-precipitins. The rate of response may be slow, however, and many repeated injections of large numbers of streptococci may be necessary before detectable antibody can be demonstrated. It is of considerable significance that cases of scarlet fever appeared to produce carbohydrates precipitin with somewhat greater frequency than those who had rheumatic fever. This tends to minimize consideration of the rôle of sensitization to group-specific streptococcal polysaccharide as a possible mechanism in the development of the rheumatic state. Since this antibody appeared in only about one-third of the cases of bacteriologically proved streptococcal pharyngitis, failure to detect its presence obviously does not eliminate the possibility that an individual has had recent experience with the *Streptococcus.*

The demonstration of a relatively high incidence of carbohydrate-precipitating antibody in a number of infectious diseases produced by agents completely unrelated, so far as is known, to the beta-hemolytic *Streptococcus* is interesting and important. The frequent occurrence (41 per cent) of this precipitin in scabies and its demonstration in 15 per cent of individuals with syphilis or chicken pox raises some interesting problems. It is entirely possible that the patients with these diseases who exhibited this antibody had also had a recent streptococcal infection, although no evidence of this was present at the time they were studied. If the anti-C precipitin persists in the blood for a long time, its presence in individuals with scabies, for example, might be explained on the ground of streptococcic invasion months or years prior to the present examination. The possibility that *Sarcoptes scabei* may possess a polysaccharide similar or closely related to that of *Strep. pyogenes* also merits some consideration, although there is no evidence in support of such a speculation. The demonstration of carbohydrate precipitating antibody in the blood of patients, without clinical or laboratory evidence of recent experience with the beta-hemolytic *Streptococcus,* cannot be used to prove or disprove the diagnosis of acute streptococcosis or rheumatic fever.

Conclusions

1. A modified method for the purification of the group-specific polysaccharide of Group A *Streptococcus* is described.

2. The incidence of precipitin for streptococcal carbohydrate is somewhat lower in rheumatic fever than in uncomplicated streptococcal pharyngitis.

3. Only about one-third of patients with bacteriologically proved scarlet fever develop anti-polysaccharide antibody.

4. Precipitating antibody for streptococcal carbohydrate is frequently demonstrable in the absence of streptococcic infection.

5. Absence of anti-C antibody does not rule out the presence of active streptococcic disease or rheumatic fever.

6. Hypersensitization to group-specific polysaccharide is of no significance in the pathogenesis of the rheumatic state.

References

1 Bruce, J. G. and Caswell, M.: Precipitin reactions in rheumatoid arthritis. J. Laborat. Clin. M., 1940, *26*, 464.

2 Chasis, H. and McEwen, C.: Precipitin reactions between hemolytic streptococci of various groups and immune and rheumatoid arthritic sera. J. Immun., Balt., 1936, *31*, 439.

3 Coburn, A. F. and Pauli, R. H.: Studies on the relationship of streptococcus hemolyticus to the rheumatic process. III. Observations on the immunological responses of rheumatic subjects to hemolytic streptococcus. J. Exp. M., 1932, *56*, 651.

4 Dawson, M. H., Olmstead, M., and Jost, E. L.: Agglutination reactions in rheumatoid arthritis. III. Comparison of agglutinins and precipitins for streptococcus hemolyticus in rheumatoid arthritis. J. Immun., Balt., 1934, *27*, 355.

5 Dawson, M. H. and Olmstead, M.: Agglutination and precipitation reactions in rheumatoid arthritis with hemolytic streptococci groups A to G. Proc. Soc. Exp. Biol. M., N. Y., 1936, *34*, 80.

6 Lancefield, R. C.: The antigenic complex of streptococcus hemolyticus. II. Chemical and immunological properties of the protein fractions. J. Exp. M., 1928, *47*, 469.

7 Lancefield, R. C.: The antigenic complex of streptococcus hemolyticus. III. Chemical and immunological properties of the species-specific substance. J. Exp. M., 1928, *47*, 481.

8 McEwen, C., Bunim, J. J., and Alexander, R. C.: Bacteriologic and immunologic studies in arthritis. J. Laborat. Clin. M., 1935, *21*, 465.

9 Neil, M. S. and Hartung, E. F.: Precipitins for streptococcus hemolyticus in rheumatoid arthritis sera. J. Laborat. Clin. M., 1936, *22*, 881.

10 Rantz, L. A. and Randall, E.: Antibacterial precipitating antibodies in group A hemolytic streptococcus sore throat. Am. J. Med., 1947, *2*, 551.

11 Seegal, D., Heidelberger, M., Jost, E. L., and Lyttle, J. D.: Precipitins against fractions of streptococci in hemolytic streptococcus disease, glomerular nephritis, rheumatoid arthritis and S. viridans endocarditis. Proc. Soc. Exp. Biol., N. Y., 1932, *30*, 582.

INFANT DIARRHEA IN FINLAND*†

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Pediatric and public health history has demonstrated a brilliant and generally successful struggle against diarrheal disease. Particularly in the twentieth century, many countries all over the world with marked variations in geography, climate, and culture have experienced a progressive and notable decline in infant mortality due to diarrhea. The extent of this decline in some areas has been more than 90 per cent in the last thirty years, so that, for example, infant mortality in New York City from all causes in 1940 was lower than the infant mortality from diarrhea alone in 1910. New York's experience is cited only because of size and relative accuracy of figures; the same picture, with only quantitative variations, has been reproduced in many other cities and many other countries.

Finland was one which had experienced a progressive decline and it was therefore a matter of serious concern to Finnish public health workers and pediatricians to observe, in the decade beginning in 1940, an apparent reversal of the trend and a recurring high incidence of diarrheal disease and mortality among infants. The reversal was first observed at the outbreak of war in 1939 but continued, and even accelerated in the fall of 1944, after the end of the war. Because of this, the government of Finland requested the assistance of the World Health Organization in analyzing the problem and in making recommendations for its solution. The writer§ was asked by the Organization to visit Finland in the summer of 1950 for these purposes.

Statistical Background

Finland is a country of some 4,000,000 people, occupying an area of 350,000 sq.km. (135,000 sq.mi.) with some 75 per cent of the population living in rural areas. There are ten provinces (lääni), of which the largest in area is the northernmost, Lapland. Helsinki, the capital, is in Uusimaa province and has about 400,000 habitants, comprising 10 per cent of the total population of the country, or 40 per cent of the urban population.

The basic political unit is the "kunta" of which there are at present something over 550. A few additions occur each year, when a kunta becomes

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* From the Division of Education and Training, Pan American Sanitary Bureau, Regional Office for the Americas of the World Health Organization, Washington 6, D. C.

† Based on a report to the Regional Office for Europe, World Health Organization, Document MH.859-50. Grateful acknowledgment is made to the World Health Organization and the State Medical Board, Finland, for permission to use the material.

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Received for publication March 4, 1953.

so large in population that it seems desirable to split it into smaller geographic units. There are three basic classifications of kunta, cities (kaupunki), small towns (kauppala), and rural kunta (maaseutu kunta). The distinction between these units is largely historical and evolutionary and there is no clear-cut population differential. In Uusimaa province, for example, there is a rural kunta with a population of 26,000, a small town with a population of 8,000, and a city with a population of 4,000.

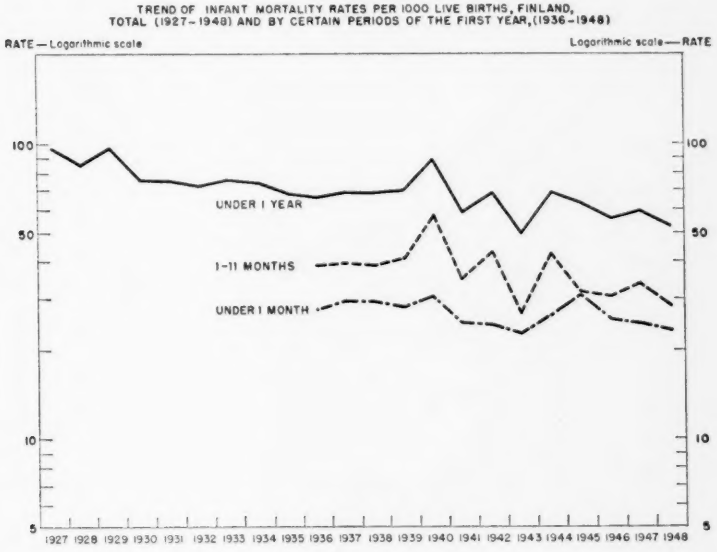
TREND OF INFANT MORTALITY RATES PER 1000 LIVE BIRTHS, FINLAND,
TOTAL (1927-1948) AND BY CERTAIN PERIODS OF THE FIRST YEAR, (1936-1948)
RATE — Logarithmic scale
Logarithmic scale — RATE
100
50
10
5
UNDER 1 YEAR
1-11 MONTHS
UNDER 1 MONTH
1927 1928 1929 1930 1931 1932 1933 1934 1935 1936 1937 1938 1939 1940 1941 1942 1943 1944 1945 1946 1947 1948

FIG. 1.

Diarrheal disease in infancy is closely related to infant mortality, which indeed was the compelling reason for initiating this investigation. Figure 1 shows the decline in infant mortality in Finland by age. Except for peaks during the two war periods, the trend has been downward, yet the curves for the first month and age one to eleven months have been similar. In total magnitude, decline has been slower than in countries with the lowest infant mortality which have now reached about half the Finnish rate. This is in marked contrast to the U.S.A., for example, where thirty years ago mortality after the first month constituted two-thirds of the total but now constitutes only one-third (Fig. 2). Thus, the slowness in decline in infant deaths in Finland seems related to persistence of mortality in the period of infancy between the second and twelfth months rather than in the newborn, the period in which most fatal cases of so-called virus diarrhea have been

reported. Furthermore, of all the diseases causing mortality in the U.S.A. in the second to twelfth month, the most rapid decline has been in infectious diarrhea.

Figure 3 compares the trend of deaths for diarrheal disease, including dysentery, in the U.S.A. and Finland. From a relatively comparable picture

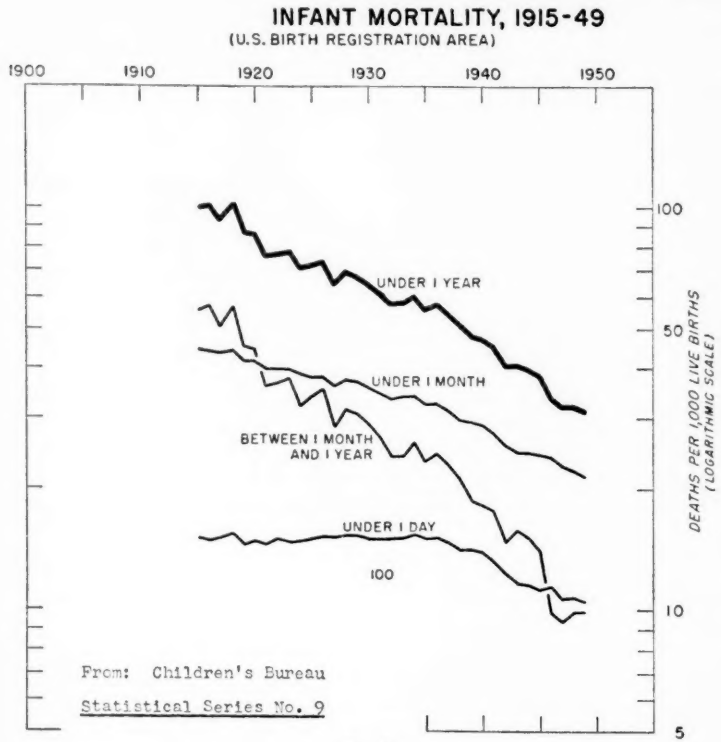

FIG. 2.

in 1936, the U.S.A. has continued to move downward while Finland has gone irregularly upward. The first increase is related to the war, but the largest increase did not take place until after the war. This persistent reversal of previous improvement was a cause for the greatest concern. Geographically, the rates for diarrheal disease differ considerably throughout Finland. The highest rate, almost twice those of Helsinki and surrounding area, exists in the northernmost province, Lapland, which has about half the area of the country but a population of about 150,000. Lapland lies

almost completely above the Arctic Circle. In the U.S.A., rates for diarrheal disease are consistently higher in the southern states, with relatively warmer climate. The determining factor, however, seems to be socio-economic rather than geographic; high rates are found also in the northernmost counties of Maine and Vermont, where a large part of the population lives in relatively poorer conditions. The latter factor applies to Lapland, which has perhaps

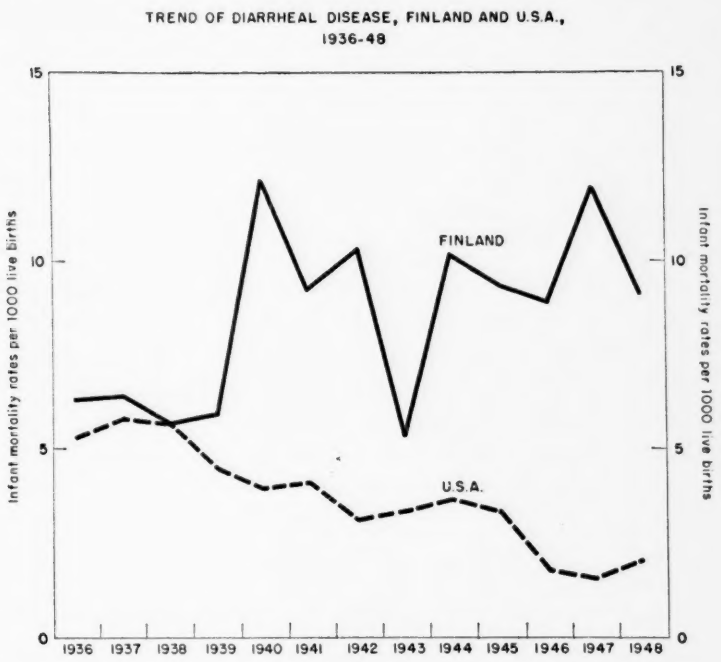
TREND OF DIARRHEAL DISEASE, FINLAND AND U.S.A., 1936-48
Infant mortality rates per 1000 live births
Infant mortality rates per 1000 live births
15
10
5
0
FINLAND
U.S.A.
1936 1937 1938 1939 1940 1941 1942 1943 1944 1945 1946 1947 1948

FIG. 3.

the lowest economic status of any of the Finnish provinces. This type of consistent geographical difference has not been observed in relation to "virus" diarrhea, epidemics of which are more often reported from well-organized and more highly developed communities.

Seasonal distribution is illustrated in Figure 4. Here the data are presented in three-year periods from 1936 to 1948. It is to be noted that both in the period of lowest incidence, such as 1936-38, and the period of highest incidence, 1945-48, there is the same general pattern of sharp increase in the summer months. This has been the classical picture of

bacterial diarrhea in years of high incidence. As incidence of the disease declined, seasonal differences have disappeared almost completely.

One of the phenomena of the decline of infant mortality in the U.S.A. has been the relative change in urban and rural areas. When infant mortality was high, the rural rates were substantially lower than urban rates. Improvement in the latter, however, was more rapid. This was thought to

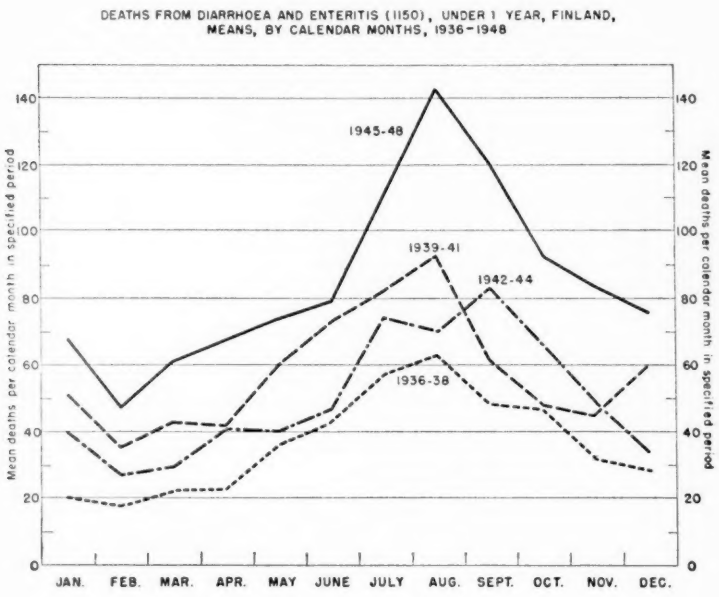

FIG. 4.

be associated with the more rapid improvement of health services for infants as well as betterment of general sanitation in cities. In Finland, as seen in Table 1, urban rates were lower in the period 1937-39, but since then have tended to increase so that in 1946-48 the urban rates significantly exceeded the rural rates. This would suggest greater retrogression in urban conditions.

Etiologic Considerations

The preceding material is interpreted as being compatible with classification of the diarrhea under study as essentially what has been previously reported, both in Finland and the rest of the world, under the lay term "summer diarrhea" or "cholera infantum." While it is thought that nutritional factors and other unknown factors, perhaps, enter into this disease,

the predominant cause is assumed to be infectious and bacteriological. Studies of Hardy and Watt,[4] for instance, have shown that in areas with high prevalence of diarrhea, meticulous hospital investigations and community surveys with newer laboratory techniques show a high proportion of isolations of Shigella and Salmonella. This by no means, however, accounts for all of the cases observed. Recently other organisms, such as the paracolon bacillus, Pseudomonas aeruginosa, and Proteus morgani, as well as pathogenic strains of E. coli, have been implicated. A recent, long term study[5] in the Premature Unit at Charity Hospital in New Orleans casts doubt upon the significance of the first three of these, while the "pathogenic"

TABLE 1

TREND OF MORTALITY RATES FROM DIARRHEA AND ENTERITIS PER 1,000 LIVE BIRTHS, CHILDREN UNDER ONE YEAR

FINLAND, THREE-YEAR PERIODS FROM 1937-48

Urban	*1937–39*	*1940–42*	*1943–45*	*1946–48*
Births	37,035	44,207	57,330	75,838
Deaths	185	465	527	872
Rate	5.0	10.5	9.2	11.5
Rural				
Births	190,143	172,879	193,986	246,164
Deaths	1,166	1,786	1,545	2,320
Rate	6.1	10.3	8.0	9.4

colon bacillus (Kauffmann strains 0-55 and 0-111) have been implicated chiefly in nosocomial epidemics. Even there it is possible for sudden outbreaks to have an undetermined origin, although the method of transmittal may be relatively clear. Such an outbreak was reported from Charity Hospital by the author in 1950.[6] Furthermore, it appears that in Finland the chief problem has been an increased prevalence of endemic diarrhea rather than an epidemic or series of epidemic outbreaks.

One of the reasons for the suggestion by Finnish investigators that a new virus might be implicated was the small number of positive isolations of Shigella and Salmonella on hospitalized cases. The possibility of pathogenic strains of E. coli was also considered but these, too, were not being found in the laboratory in significant numbers. Although in the largest children's hospitals meticulous bacteriological examinations were being undertaken, several shortcomings in techniques existed. The time interval from collection of specimen to inoculation of media was often undesirably long and some of the newer, more highly selective media, such as S.S.Agar, were not available because of economic difficulty. The identification procedures appeared unnecessarily complicated, with lack of a preliminary

screening solid medium, such as triple sugar-iron-agar, to facilitate the picking of multiple suspicious colonies. It seemed likely that improvement in these and other technical details[1, 2, 8] would increase the number of positive results. On the other hand, it appeared perhaps more important to determine the likelihood that the difficulty was related to infection and to elucidate whether or not new factors were responsible for the observed increased prevalence of the disease.

Factors Contributing to Increased Prevalence

While it is obviously desirable to intensify efforts to find specific etiologic agents, it is not necessary to wait for this to undertake measures designed to reduce the incidence and mortality of diarrheal disease. The possible influencing factors considered of importance after investigation were basically three:

1. *Living conditions and general sanitation*

While Finland is not a wealthy country, homes are, in general, solidly constructed and visits to many showed obvious evidence of considerable interest in keeping the household neat. Overcrowding, however, was generally present. This was related not only to the general disruption of wartime movement, but also to the territorial changes involved in postwar treaties. Two and three families were said to be occupying space hardly sufficient for one. This apparently has been true not only in cities, but also in rural areas. It was not uncommon for one or more families to occupy a single room and to keep their water supply in a bucket filled by periodic visits to a nearby well. During the war and the immediate postwar period, there were great shortages in soap, and hot water was scarce. Little emphasis was given to the importance of hand cleanliness, particularly in relation to possible transmission of disease. During the summer months, flies and gnats are seen throughout the country, particularly in Lapland. Screens are almost unheard of and are not seen even in the houses of relatively well-to-do people.

There is a considerable absence of facilities for home refrigeration. This is based in part on the lack of need for such refrigeration during most of the year. Electric refrigeration is rare and although ice is plentiful during the winter, no year-round domestic ice industry has developed.

Relationship of houses and living conditions to health has received increasing understanding in recent years. Improvement, however, requires more than public health action. On the other hand, it should be possible to compensate to some extent for overcrowding and inadequate sanitation through further emphasis on personal hygiene, particularly hand washing for those caring for children. This group may be easily reached through the child health services and public health nursing service discussed below.

An interesting sidelight on living conditions and community mores is

the general Finnish devotion to "sauna." Sauna is the Finnish bath, considered almost a ritual for young and old alike. Rare, indeed, is the Finn who does not visit sauna at least once a week. Sauna is characterized by exposure for a period, in a small room, to an extremely hot, essentially dry, atmosphere, followed, if possible, by a swim or rolling in the snow, and then by a soap and water scrub. There are both community and individual saunas and the institution is naturally conducive to a fairly high measure of personal cleanliness. The high temperature is produced by heating stones on which, from time to time, water is thrown. The heat of the room is usually so great, however, that the water is converted immediately into live steam and no vapor is ever visible. The first sauna which the writer visited,

TABLE 2

RELATION OF DIARRHEAL DISEASE TO TYPE OF FEEDING, 1949

(Data of Ylppö, Hallman, *et al.*)

	Breast feeding	*Powdered milk mixtures*	*Cow's milk mixtures*
Children	1,256	1,208	1,294
Cases	57	104	233
Rate Percentage	4.6	8.7	19.8

the day of arrival in Finland, had an air temperature recorded on an accurate mercury thermometer of 94° Centigrade at the time of entrance, and 102° Centigrade at the time of leaving, about fifteen minutes later! Such a heat would, of course, be absolutely insupportable if it were not for the essential absence of moisture.

2. Milk supply and infant feeding techniques

In 1949, Professor Ylppö and his group at the Children's Clinic in Helsinki studied the relationship of diarrheal disease to type of feeding in four selected cities. The data are given in Table 2 and indicate clearly the advantage of breast feeding or, in the absence of breast feeding, of a powdered rather than a fresh milk. No figures are available concerning the prevalence of breast feeding in Finland at the present time, but it appears that the same type of general decline has been observed in Finland as in other countries. While public health nurses have actively attempted to encourage breast feeding, their efforts have not been too well coördinated or too successful. The powdered and evaporated milk industry is poorly developed and consequently the cost of other than fresh milk is substantially greater than fresh milk.

Throughout Finland, cow's milk is distributed very widely. It is reported that the per capita consumption of cow's milk in Finland is one of the high-

est in the world. Quality of milk production on the farms varies, of course, but it is said to be generally acceptable. Those visited had good standards but equipment badly needed replacement, delayed by general economic difficulty. Ice is cut during the winter to keep the milk refrigerated during the summer months, until collection from the farm. Milk trucks are not refrigerated. At one large dairy, the highest bacterial counts on raw milk were observed in May and September. The explanation was offered that in May the farmers thought refrigeration was not necessary because the temperature was not high enough, and in September the ice was all used up.

Most of the milk produced falls in two classes by bacterial count: in 1949, 66 per cent was first class (less than 1,000,000 bacteria per cc.), and 25 per cent, second class (less than 5,000,000 bacteria per cc.). Milk is generally distributed in thirty- and fifty-liter cans to local milk shops where it is dispensed to the customer's own container by the use of a dipper. The can is refrigerated sometimes, but only until it is opened for the first time. Bottling of milk is essentially limited to certified raw milk (produced under more rigid conditions and with bacterial count less than 500,000 per cc.), which costs 50 per cent more than ordinary milk.

Pasteurization of milk is quite irregular, although many of the dairies do have reasonably satisfactory pasteurization equipment. Most of the veterinarians, who have a potent influence on the milk industry, believe that pasteurization has little to offer in the way of advantages and has some positive disadvantages. They believe that milk spoils more easily if not refrigerated after pasteurization, that vitamins are destroyed, and that possible change in taste may affect the milk drinking habits of the Finnish people, on which their generally good nutrition is based. Furthermore, there is said to be no bovine tuberculosis or brucellosis in Finland.

The chief deterrent to universal production of bottled milk is economic. Bottling or putting milk in cartons would raise the cost of milk about 40 per cent. It was suggested that a start on the introduction of bottled milk might be made by producing a special milk for babies with a lower fat content, say 3.0 per cent. Since most milk averages 4 per cent, the saving in fat could be used to compensate in part for the increased cost of bottling the milk. This idea is currently under discussion by leaders of the milk industry and advisers from the United Nations International Children's Emergency Fund (UNICEF), which is very actively concerned with helping the milk industry in Finland to reach higher standards.

There seems little doubt that universal pasteurization of milk is desirable, but this needs to be part of a program which assures safety of the milk when delivered to the customer. This entails better refrigeration and handling in the milk stores, as well as continued efforts towards bottling of the milk.

Preparation of cow's milk for babies in the home. There was noted a tendency to make preparation of milk mixtures over-elaborate through requiring addition of special cereal water as the diluent. Furthermore, in

the preparation, the milk is brought just to a boil and the heat is turned off. This is in part in an attempt to preserve Vitamin C, for which, however, cow's milk is not a satisfactory source in any event. It was recommended that plain water be used for dilution and that the milk be actually boiled.

3. *Organization of health services for children**

Finland is fortunate in having an extensive organization of public health centers. An increase in the number of these centers was an important part of postwar reconstruction in Finland and at the end of 1949 there was a total of 643 main health centers and 2,109 sub-centers. Most of the main health centers, in addition to the rooms for clinics and general operation, have living facilities for a public health nurse, a midwife, and a "home-maker," who is essentially a visiting housekeeper. These health centers are very pleasant, well constructed, and the provision of satisfactory living quarters is a strong inducement for girls to enter these professions and to accept employment in more or less isolated places.

The public health nursing service is one of the prides of Finland and one to be envied elsewhere. Public health and government authorities in postwar planning reached the conclusion that whereas one public health nurse per 5,000 of the population was used as a standard in most technically developed countries (although it should be pointed out that this figure has not been reached for the U.S.A.), in view of the higher morbidity and mortality rates in Finland, a higher standard was necessary despite economic stringency. A goal of one nurse for every 4,000 population was therefore set. Provision has been made in the budget to achieve this figure, and by the end of 1949 there were, actually working, nurses in the proportion of one for every 4,350 of the population. In addition, there were 164 visiting nurses doing bedside work and 317 religious deaconesses, who are registered nurses and give a large proportion of the nursing care to the aged and chronically ill. Furthermore, the public health nurses have all had a three-year basic nursing course and a year of specialized public health training. It is not unreasonable to assume that, had there not been so extensive a nursing service, the factors responsible for increase in diarrheal disease would have produced a much more serious situation.

Certain shortcomings were to be noted, however, in the health services for children. There were no specialized consultants to assist the Director of Public Health Nursing Services in such important fields as maternal and child health or communicable disease work. Transportation was a difficult problem. No public health nurse had a car; most had their own bicycles, but depended on walking and skis in the winter.

The medical organization of maternal and child health work was far behind the nursing. While there was an extensive system of child health

* It is to be noted that the data in this section, as in the rest of the report, are as of the time of the investigation, July 1, 1950.

conferences, they were generally run by local practitioners without central supervision or guidance. There was no Division of Maternal and Child Health in the State Medical Board and no pediatrician in a position of responsibility toward the services for children. Establishment of a real Division of Maternal and Child Health appeared an essential step to give more direction and coördination of efforts in the Child Health Conferences and also to allow more coördinated guidance for the nurses' efforts in their work with children.

Summary

Review of official statistics indicates that Finland has had during the last decade a recrudescence of diarrheal disease in infants. The clinical pattern of the disease, its age selection and its geographic distribution are compatible with recurrence of a known disease or diseases rather than development of a new one.

Laboratory investigation has failed to identify any characteristic pathogens, but improvement in methods of collection and examination of specimens is possible.

Factors possibly contributing to the spread of the agent or agents responsible for the disease include: crowded living conditions and inadequate sanitation; probable decrease in breast feeding; difficulties in production and distribution of cow's milk and in methods of preparation for infant feeding in the home; imbalance in child health organization and pediatric guidance of public health nursing activities for children.

These factors are susceptible to a coördinated public health program, including child health services and health education, as well as milk conservation, even in the absence of specific knowledge of the responsible etiologic agent.

References

1 Edwards, P. R.: Simplified serologic identification of Salmonella cultures. Com. Dis. Center Bull., 1950, *9* (No. 5), 9.

2 Ewing, W. H.: Simplified serologic identification of Shigella cultures. Com. Dis. Center Bull., 1950, *9* (No. 5), 15.

3 Ewing, W. H. and Edwards, P. R.: Selection of Salmonella and Shigella cultures for serologic classification. Com. Dis. Center Bull., 1950, *9* (No. 5), 1.

4 Hardy, A. V. and Watt, J.: Studies of the acute diarrheal diseases XVIII. Epidemiology. Pub. Health Rep., Wash., 1948, *63*, 363.

5 Watt, J., Maupin, E., Hemphill, E., and Wegman, M. E.: Continued presence of Proteus, Paracolobactrum and Pseudomonas in a large premature infant nursery without an epidemic of diarrhea. Read by title before the American Pediatric Society, Atlantic City, N. J., May 1951.

6 Wegman, M. E.: An epidemic of diarrhea among breast fed newborn infants. J. Am. M. Ass., 1951, *145*, 962.

ANTIBIOTIC THERAPY*

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The advent of each new antibiotic is not an unmixed blessing. The advantage of acquiring another agent whose range of antibacterial activity parallels that of already existing antibiotics is somewhat offset by the necessity of re-evaluating the known in the light of the new unknown. And involved is not just the effectiveness of one agent versus another, but the relative cost, potential toxicity, and inherent capacity to induce resistance of each.

The following is a brief summary of the present status of antibiotic therapy. In addition to a table listing the drugs of choice for systemic use against the micro-organisms most commonly involved in clinical infections, it includes a brief discussion of the principles underlying antibiotic treatment based on experimental findings. It is neither a review of the literature nor a handy compendium of drug dosages but rather represents current experience and thinking in a field in which the author has been working for a number of years.

Systemic Use

The antibiotics of choice for the treatment of infections are presented in the table. When two antibiotics are of equal effectiveness, they are so listed.

In general, it will be noted that penicillin is still the drug of choice in most infections caused by gram positive organisms, both coccal and bacillary, whereas infections caused by gram negative bacteria are best treated by streptomycin, aureomycin, or terramycin. The group of Neisseria and the spirochete of syphilis represent notable exceptions to this generalization, penicillin being still the most effective antibiotic against these gram negative organisms.

Chloromycetin (chloramphenicol), like aureomycin and terramycin, is a broad spectrum antibiotic highly effective in a variety of infections but because of its alleged tendency to produce blood dyscrasias it has dropped perhaps unjustifiably into third place (effective but perhaps toxic) in most instances. Where indicated in severe infection, however, it should be used, provided the risk to life from the infection outweighs the risk of possible toxicity.

Polymyxin B is one of the most active antibiotics *in vitro* against gram negative bacteria but because of its potential nephrotoxicity, its systemic use

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Received for publication February 22, 1953.

has been severely limited, possibly unduly so. Recent reports would indicate that polymyxin was not only the drug of choice in a series of cases of enteric and urinary tract infections, notably those due to *Pseudomonas aeruginosa*, but that in the children treated, it showed very little evidence of renal toxicity. This is confirmed by the author's experience in observing a small series of premature infants with pseudomonas infections. Polymyxin was not only efficacious in treatment, but in no instance did it produce any renal toxic effects.

Neomycin and bacitracin are poorly absorbed agents that have not been recommended for systemic use because of their toxicity. Yet they too, like polymyxin, are beginning to find wider favor for oral use against certain enteric infections because, taken orally, neither one produces significant side reactions. Neomycin has been found to be an excellent intestinal antiseptic since it is quite active against both gram positive and gram negative bacteria. Bacitracin exerts its effect primarily against gram positive enterococci and clostridia.

Erythromycin, one of the newer antibiotics, shows a wide range of activity against many gram positive pathogens and a few gram negative ones. It seems relatively non-toxic and since it is active against many staphylococci and streptococci that are resistant to other antibiotics, it should prove to be a valuable addition to the broad spectrum antibiotics.

Streptomycin still leads the field as a tuberculostatic drug, and it combines well with other antibiotics for other infections. In tuberculous infections, the combined use of streptomycin and para-aminosalicylic acid or nicotinic acid derivatives or both is not only desirable therapeutically but tends also to reduce the hazard of eighth nerve damage (by reducing the dosage of streptomycin) and the risk of development of streptomycin resistance.

In severe infections, combinations of antibiotics are sometimes indicated. In subacute bacterial endocarditis, for example, an adequate combination of penicillin and streptomycin (gram positive infecting organism) or streptomycin and aureomycin (gram negative infecting organism) may turn the tide when any of these agents alone will not. Unfortunately, combinations of antibiotics may result in antagonistic as well as synergistic effects. If penicillin, streptomycin, neomycin, and bacitracin are considered as one group (A) and aureomycin, chloramphenicol and terramycin as another (B), then the members of group A tend to act synergistically towards each other but antagonistically towards members of group B. Group B agents tend to potentiate each other but to behave antagonistically if combined with agents in group A. So, although the last word has not been said, it would seem advisable to avoid combinations of antibiotics when one alone is effective and to use only combinations that have been *proved* to be effective against infections clinically.

TABLE 1

ANTIBIOTIC THERAPY: CHOICE OF DRUG

Type of infection	*Penicillin*	*Streptomycin*	*Aureomycin*	*Chloromycetin*	*Terramycin*	*Erythromycin*
Streptococcal infections:						
1. Beta hemolytic	1	2	2	3	2	2
2. Alpha hemolytic	1	2	2	4	2	5
3. Non-hemolytic	1	5	2	5	2	
4. All enterococci	2	2	1	3	2	5
Staphylococcal infections	1	2	2	3	2	2
Pneumococcal infections	1	2	2	3	2	2
Neisserian infections:						
1. N. meningitidis	1S	6	2	3	2	5
2. N. gonorrheae	1	2	2	3	2	5
Diphtheritic infections	1A	5	2	5	5	4
Tuberculous infections	6	1PAS	5	5	5	
Anthrax infections	1	6	2	5	4	5
Clostridial infections:						
1. Gas gangrene	1	6	2	4	2	4
2. Tetanus	1	6	6	5	5	4
Gram-Neg. bacillary infections:						
1. E. coli	4	2	1	3	1	6
2. Kl. pneumoniae	5	1	1	3	2	6
3. Proteus	2	1S	5	3	2	6
4. A. aerogenes	6	2	1	3	2	6
5. Ps. aeruginosa	6	1S	2	4	2	6
Salmonella infections:						
1. S. typhosa	4	4	2	3	2	6
2. Others (Para. A + B)	5	4	4	3	4	6
Shigella infections	6	2	2	3	1S	5
Pasteurella infections:						
1. P. pestis	6	1	4	4	5	
2. P. tularense	6	1	1	3		
Brucella infections:						
1. Br. melitensis 2. Br. abortus 3. Br. suis	6	2	1	3	2	5
Cholera	5	2	5	4	5	5
Hemophilus infections:						
1. H. influenzae meningitis	4	1S	1S	3	2	4
2. H. pertussis	6	4	4	4	4	4
3. H. ducreyi	6	1	2	3	2	
Treponema infections						
1. Pallidum	1	6	2	3	2	
2. Pertenue	1	6	2	3	2	

1 First choice
2 Second choice
3 Effective but potentially toxic
Blank—Not evaluated
4 Effective in vivo but clinical value not established
5 Effective in vitro
6 Not effective
S—Sulfadiazine also
A—Antitoxin also
PAS—Para-amino salicylic acid also

Topical Use

Neomycin topically is the antibiotic of choice against nearly all pyogenic infections of the skin, mucous membranes, and eyes. It has a very wide range of activity against both gram positive and gram negative organisms and it rarely induces hypersensitivity.

Bacitracin has an antibiotic range roughly similar to penicillin but, unlike penicillin, it maintains its activity in mixed infections and it is essentially non-sensitizing. It is an excellent agent for treating local skin infections caused by staphylococci and streptococci.

Polymyxin B is probably the drug of choice for topical use against pseudomonas infections.

Broad spectrum antibiotics, although effective topically, should, by and large, be reserved for systemic use in order to reduce the risk of inducing hypersensitivity.

Prophylactic Use

As the range of effectiveness of antibiotics against infections has been increased by the discovery of new agents, so too has the use of antibiotics in the prophylaxis of infections.

Topically, penicillin is of value for the routine prophylaxis of gonorrheal opthalmia.

In closed communities, penicillin, given by mouth, is useful in the prevention of hemolytic streptococcal or meningococcal infections.

Small daily doses of oral penicillin have been found to be very effective in the prevention of recurrences of rheumatic fever. (The same is true of sulfadiazine.) In the rheumatic subject, the intramuscular administration of procaine penicillin before and after the extraction of teeth, the removal of tonsils, or for that matter any operative procedure around the nose or throat, aids materially in preventing the development of subacute bacterial endocarditis.

The risk of infection after certain "clean" operations, for example, corrective orthopedic surgery, is much decreased by the prophylactic use of antibiotics. In established infections in which surgical interference is indicated, the proper use of antibiotics greatly facilitates the control of the postoperative spread of micro-organisms.

Use and Misuse

If chosen and administered intelligently, antibiotics can be depended upon to produce maximal therapeutic effects with minimal adverse side reactions. In addition to selecting with care the drug of choice for each particular infection, it is necessary to keep in mind a few basic principles governing the administration of these agents.

Antibiotics should be given in large enough doses and for a long enough time to control the infection. Inadequate treatment is worse than no treat-

ment at all because it permits the emergence of drug resistant variants of the infecting micro-organism. With certain antibiotics, such as streptomycin, resistant variants are prone to appear rapidly. Even with those agents less apt to give rise to resistant forms, the problem is always potentially there and as time goes on, more and more micro-organisms are appearing which seem to be resistant on primary isolation to certain antibiotics. Cross resistance, too, has become an ever-increasing problem. Organisms readily develop mutual cross resistance to aureomycin, terramycin, and chloramphenicol, and there is some evidence to show that resistance to streptomycin is paralleled by resistance to neomycin. In treating an infection that has become resistant to one antibiotic, the possibility of cross resistance should be kept in mind in choosing another agent.

Overly prolonged therapy is just as unwise as inadequate therapy because it greatly increases the incidence of untoward reactions. In general, if definite clinical improvement is not evident within two to three days, the value of further therapy with a given antibiotic should be seriously questioned and sensitivity tests of the infecting organism should be run against this and other antibiotics before therapy is resumed.

This last statement must be qualified by a knowledge of the natural history and pathological nature of the infection. Infectious processes of soft tissues which are of short duration generally clear up quickly. Those in bone, in thrombi, and in certain other tissues require longer exposure to the antibiotic agent. It is also important to know whether the antibiotic has a bactericidal or a bacteriostatic action against the agent causing the infection. If the action is bactericidal, the therapeutic response is generally quite prompt; if bacteriostatic, the response may be slower and treatment must be more prolonged.

One final word. The indiscriminate use of antibiotics cannot be too strongly condemned. "Wholesale" treatment with a single antibiotic or "shotgun" therapy with combinations of antibiotics is bad medicine. No antibiotic, no matter how innocuous, is completely safe from the standpoint of potentially undesirable side-effects. One has only to think of the allergenicity of penicillin or the gastro-intestinal concomitants of the broad spectrum antibiotics to know that these agents are not brand names for sugar coated pink pills. And yet one is constantly aware of the fact that antibiotics are being prescribed right and left without regard to specific indications or, worse, with no indications at all.

Whatever dubious psychological advantage there may be in treating a cold with penicillin, it is surely more than offset by the fact that penicillin is totally ineffective against cold viruses at best and is a sensitizing drug to boot.

Antibiotics should prove to be of continuing value in the reduction of time lost from illness and in preventing needless deaths, if they are used with care and good judgment.

THE COMPARATIVE ACTION OF DICUMAROL AND OF PHENYLINDANEDIONE ON THE COAGULASE REACTING FACTOR AND ON PROTHROMBIN*

MORRIS TAGER†

Recent studies[8] have established the presence of a factor ("activator," Coagulase-reacting factor, or CRF) in plasma which is required for the coagulation of blood by staphylocoagulase. Maximum CRF activity resides in the globulin fractions of plasma, and separation from prothrombin is best achieved by repeated Seitz filtration.[10] The filtration involves different rates of adsorption of prothrombin and CRF,[12] so that when the filtrate is essentially devoid of prothrombin it still may possess substantial CRF activity. Efforts to identify CRF with factors at present known to participate in physiological clotting of blood have been unsuccessful,[10, 11] and the hypothesis has been proposed that the coagulase-CRF reaction bypasses the currently accepted pathways functioning in physiological clotting. The many points of resemblance between prothrombin and CRF, as well as the differences, have been discussed elsewhere.[8, 11] Duthie and Lorenz[3] find it tempting to consider prothrombin and CRF as identical. They point to similarities in physical properties, parallel losses of both prothrombin and CRF when prothrombin is converted to thrombin, to localization in comparable fractions of plasma, and to the finding that the concentration of both prothrombin and CRF decrease "almost *pari passu*" following dicumarol administration in rabbits. To explain the separation of CRF from prothrombin by Seitz filtration, they suggest that this procedure may not actually remove prothrombin, but that the physical structure of prothrombin is so altered that it can no longer be converted to thrombin while it continues to function as a coagulase "activator."

The observations of Duthie and Lorenz that dicumarol administration depresses CRF activity have been confirmed in this laboratory, both in rabbits and in patients undergoing dicumarol therapy for thrombotic disease. On the other hand, Miale[8] failed to note any effect on CRF levels of plasma of 21 patients on a dicumarol regime. The present study was undertaken to resolve this conflict, particularly since it seemed possible that a detailed quantitative analysis of the effect of dicumarol and related compounds on

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Received for publication March 12, 1953.

prothrombin and on CRF might contribute to a better understanding of the relation of these factors to each other.

Materials and Methods

Coagulase—a water clear, purified product prepared from *Staphylococcus aureus* #104, as previously described.[11]

Fibrinogen—Armour's bovine Fraction I, used in a concentration of 300 mgm. per cent in buffered saline at pH 7.2.

Plasma. Rabbit plasmas were collected by venipuncture in an excess of sodium citrate, care being taken to avoid clotting and to maintain a constant citrate to plasma volume ratio. Plasma dilutions were made in physiological saline with 2 mgm. of bovine albumin (Armour's Fraction V) per ml.

General procedure for the titration of CRF. To obtain the reference 100 per cent standard, plasma samples were obtained from each rabbit before drug administration, dilutions of 1:100, 1:200, 1:400, and 1:800 were prepared in saline-albumin. To 0.2 ml. lots of plasma were added 0.2 ml. of fibrinogen in pyrex test tubes 7 x 12 mm. in size. Two-tenths ml. of coagulase were forcibly blown into each tube at the instant of starting the stop watch. The tubes were removed from the 37° C. water bath and continuously observed with a 2X magnifying lens using a constant fluorescent light source. The appearance of the first wisp of fibrin, or of definite flocculation, was taken as the clotting time. Titrations were carried out in duplicate, and checks within 3-5 seconds generally obtained at the more concentrated ranges of plasma. The clotting times were plotted arithmetically, and the per cent CRF activity of post-drug plasma samples was computed from these curves. In the titrations of post-drug samples, dilutions of 1:100 and 1:200 only were tested. Controls of coagulase and fibrinogen only, as well as of plasma and fibrinogen, were simultaneously carried out, and remained negative.

Thromboplastin. Acetone extracted rabbit brain was prepared, or the commercially available Bacto-thromboplastin (Difco) was used.

Bovine plasma. Fresh beef plasma, obtained from the slaughter house, was filtered five times through EK Seitz pads. No clotting occurred upon the addition of thromboplastin and calcium within 20 minutes. An equal volume of 0.6% imidazol buffer was added, and the plasma further diluted with an equal volume of distilled water, the final pH being adjusted to 7.2.

Prothrombin determination. The method of Ware and Stragnell[18] was adopted with minor modifications. In essence, this is a one-stage technique involving the use of diluted plasma, and providing a constant supply of accelerator globulin and fibrinogen by the addition of prothrombin-free bovine plasma to the reaction mixture. Plotted logarithmically, a straight line generally is obtained from which the per cent activity of post-drug samples may be directly computed.

Procedure: Citrated rabbit plasma dilutions were prepared of 100, 80, 60, and 20% concentration respectively, taking the 1:10 dilution in distilled water as the initial 100% value. One-tenth ml. of the appropriate plasma dilutions were dispensed in 7 x 12 mm. pyrex tubes, 0.1 ml. of the prothrombin-free bovine plasma and 0.1 ml. of thromboplastin were added. After an incubation period of two minutes at 37° C., 0.1 ml. of 0.05 M $CaCl_2$ was forcibly blown in with a 0.1 ml. serological pipette at the instant of setting the stop watch into motion. The tubes were observed under the same conditions as described above for CRF titration, and were gently tilted at room temperature to note the first appearance of fibrin. Except at the more prolonged clotting times, duplicate checks within two seconds were generally obtained. The test differed from

the Ware and Stragnell technique in minor detail: the prothrombin-free bovine plasma was citrated rather than oxalated, and essentially freed of prothrombin by repeated Seitz filtration rather than by combined Seitz filtration and by barium sulfate adsorbtion. No heparin was added to the anticoagulant, but the higher concentration of citrate used to prevent the clotting of rabbit plasma necessitated the use of a higher concentration of calcium chloride.

Dicumarol (bishydroxycoumarin, Abbott). The tablets were ground in a mortar and pestle with propylene glycol[3] to form a homogeneous suspension, and the drug was administered into the upper leg muscles of rabbits.

Phenylindanedione (PID)—obtained through the coöperation of Dr. E. A. Sharp and Dr. J. E. Gajewski of Parke, Davis and Co. was prepared and administered similarly to dicumarol.

Mephyton (Vitamin K_1 Merck, 2-Methyl-3-phytyl-1,4-naphthoquinone). The emulsion, containing 50 mgm. per ml., was slowly administered intravenously, without untoward effect.

Rabbits. Adult domestically raised New Zealand white rabbits were used, ranging in weight from 3.09 to 5.02 kilograms.

Results

The effect of dicumarol and of mephyton on CRF and on prothrombin of rabbits. In a preliminary titration (Table 1) 7 rabbits received intramuscularly dicumarol, rabbits ♯4, 5 and 12, 10 mgm./kg. and rabbits ♯3, 10, 11, and 14, 25 mgm./kg. It is readily apparent that dicumarol is effective in depressing both prothrombin and CRF. However, in most animals there was no close correspondence in the effects produced on prothrombin and on CRF, with the reduction in prothrombin being generally more rapid and severe than in CRF. Individual differences in the response of the rabbits overshadowed the dose of dicumarol administered, since there was no consistent increase in clotting times with the higher level of the drug. Rabbit ♯12 was unique in showing no reduction in CRF and only a minimal prothrombin effect, confirmed by a subsequent titration.

Since the preliminary titration did not reveal any close parallelism in dicumarol action on prothrombin and on CRF, it was deemed of interest to compare the response of these factors to a substance like mephyton known to combat the anticoagulant effect of dicumarol and similar agents. Accordingly, after a rest period of a month, rabbits ♯4, 5, 10, and 11 were given 25 mgm./kg. of dicumarol, and at 72 hours from 25 to 35 mgm. of mephyton were injected intravenously in rabbits ♯4, 5, and 10, respectively. The results are summarized in Table 2, and expressed in both per cent of activity and in the actual clotting times obtained. Inspection of the data, especially when one compares the 144-hour post-dicumarol levels with those at the same time in Table 1, clearly indicates the effectiveness of mephyton in reversing the dicumarol effect on CRF. The prothrombin titers, however, increased far more rapidly, and the apparent increase in prothrombin content was evident even within four hours following mephyton administration.

TABLE 1

THE EFFECT OF DICUMAROL ON PROTHROMBIN AND CRF

*Hours after dicumarol**	*Per cent Prothrombin and CRF*													
	Rabbit #													
	3		*4*		*5*		*10*		*11*		*12*		*14*	
	Pr	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*
Pre-drug control	100	100	100	100	100	100	100	100	100	100	100	100	100	100
24	40	40	20	98	42	93	24	63	58	95	72	100	34	75
48	44	19	5.5	100	28	75	15	58	35	46	82	98	32	85
72	62	55	<1	15	12	40	10	40	32	20	92	105	65	98
144	50	72	4.5	50	34	33	1.5	15	18	28	77	100	68	100

* Rabbits #4, 5, and 12-10 mgm./kg. i.m.; rabbits #3, 10, 11, and 14-25 mgm./kg. i.m.

The effect of phenylindanedione and of mephyton on CRF and prothrombin times in rabbits. Phenylindanedione (PID)[4,5,9] has recently been proposed as an anticoagulant which might reduce some of the hazards of dicumarol because of more rapid recovery after oral administration. It

TABLE 2

THE EFFECT OF MEPHYTON ON PROTHROMBIN AND CRF AFTER DICUMAROL ADMINISTRATION

		Rabbit #									
		4		*5*		*10*		*11*		*7*	
Time in hours		*Dicumarol + mephyton*		*Dicumarol + mephyton*		*Dicumarol + mephyton*		*Dicumarol only*		*Bleeding only*	
		Pr	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*
Pre-drug control	%	100	100	100	100	100	100	100	100	100	100
	c.t. sec.	29	85	33	95	27	68	23.5	97	23	65
72 post dicumarol	%	7	1	1.5	75	19	14	13	54	99	100
	c.t. sec.	210	253	325	110	73	190	101	160	23.5	65
4 post mephyton, 76 post dicumarol	%	48	13	33	45	36	23	11	57	...	...
	c.t. sec.	50	169	137	136	48	142	106	155	...	...
24 post mephyton, 96 post dicumarol	%	135	40	85	50	135	68	11	49	110	90
	c.t. sec.	24	132	41	125	23	87	107	169	21.5	112
48 post mephyton, 120 post dicumarol	%	88	48	88	100	145	100	23	46	...	...
	c.t. sec.	32	125	39	95	21	68	63	177	...	...
72 post mephyton, 144 post dicumarol	%	100	85	100	100	140	87	23	31	...	...
	c.t. sec.	29	95	33	95	22.5	77	63	222	...	...

Dicumarol: 25 mgm./kg. i.m.
Mephyton: 25 mgm. i.v., rabbits #4 and 5; 35 mgm. i.v., rabbit #10.
Each clotting time—average of two titrations.

seemed of interest, therefore, to determine whether CRF is also affected by this drug, and whether this action is reversible by mephyton. Six rabbits were given PID intramuscularly in a dose of 75 mgm./kg. (Table 3). In spite of this excessive dose, parenterally administered, the reduction in prothrombin activity was not as drastic as with dicumarol, and by 60 hours,

TABLE 3

THE EFFECT OF PHENYLINDANEDIONE* ON PROTHROMBIN AND CRF

Time in hours		Rabbit # 4 Pr	4 CRF	8 Pr	8 CRF	9 Pr	9 CRF	11 Pr	11 CRF	12 Pr	12 CRF	13 Pr	13 CRF
Pre-drug control	%	100	100	100	100	100	100	100	100	100	100	100	100
	c.t. sec.	24.5	77	32.5	77	24	64	25	72	31	88	31.5	80
18	%	40	86	60	82	45	51	50	78	80	110	70	90
	c.t. sec.	36	83	45	88	39	84	44	82	36	84	39	85
36	%	30	42	42	66	36	50	33	37	75	82	55	50
	c.t. sec.	41.5	122	60	99	44	90	58.5	119	38	97	45	110
60	%	57	67	150	120	32	45	38	34	135	105	95	38
	c.t. sec.	32.5	97	24	65	48	110	52.5	129	26	86	33	126

* PID—75 mgm./kg. i.m.
Each clotting time—average of two titrations.

most of the animals were showing either rising titers or had returned to normal levels or beyond. The CRF reduction was not excessive either, was generally less marked than the prothrombin response, and was usually associated with a less rapid trend toward recovery at 60 hours. As in the experiments with dicumarol, rabbit #12 responded very poorly, with relatively slight action on prothrombin and on CRF alike.

TABLE 4

THE EFFECT OF MEPHYTON ON PROTHROMBIN AND CRF AFTER PHENYLINDANEDIONE ADMINISTRATION

		Rabbit #							
		2		*4*		*8*		*13*	
Time in hours		*PID + mephyton*		*PID + mephyton*		*PID + mephyton*		*PID only*	
		Pr	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*	*Pr*	*CRF*
Pre-drug control	%	100	100	100	100	100	100	100	100
	c.t. sec.	23.5	86	21.5	79	23	76	28.5	85
18 post PID	%	48	85	50	78	28	18	80	87
	c.t. sec.	34	92	33.5	88	56	205	32.5	93
4 post mephyton, 22 post PID	%	80	75	105	99	62	49	43	84
	c.t. sec.	26	97	20	80	33	100	43	95
24 post mephyton, 46 post PID	%	110	100	105	99	82	62	26	48
	c.t. sec.	22	86	20.5	80	26.5	90	58	130

PID—75 mgm./kg. i.m.
Mephyton—40 mgm. i.v.
Each clotting time—average of two titrations.

When mephyton was administered, in a dose of 40 mgm. per rabbit intravenously, prompt reversal of the anticoagulant effect of PID was noted (Table 4). While the number of observations was too few to permit any generalization, and allowing for the differences in the 18-hour post-PID levels of prothrombin and CRF, a fairly close parallelism of action was evident.

DISCUSSION

The data presented indicate that, like prothrombin, the coagulase-reacting factor is depressed by the administration of dicumarol and of phenylindanedione. Similarly, Vitamin K_1 (mephyton) effectively counteracts this depressing action on both prothrombin and CRF. If one adopts the

view that these anticoagulants act by interfering with the synthesis of prothrombin in the liver from Vitamin K-like precursors, then these results may be interpreted as pointing to a structural similarity between the precursors of prothrombin and of CRF. The close relationship between prothrombin and CRF is further suggested by the response of rabbit #12. In this animal, both anticoagulants exerted minimal effects on prothrombin and on CRF alike.

In no sense, however, should these data be construed as implying an identity of structure of prothrombin and of CRF. It has recently been shown that dicumarol has an effect also on prothrombin activators,[5, 7] hence its action cannot any longer be regarded as specifically interfering with prothrombin synthesis only. Even *pari passu* action of all the agents tested would not establish the identity of prothrombin and CRF, since this might represent similar action on closely related substances rather than on one factor. Actually, *pari passu* effects on prothrombin and on CRF were by no means consistently observed, even when allowances were made for differences in the sensitivity of the tests. On the contrary, frequently a sharp divergence in the prothrombin and CRF clotting times was observed. It is hoped that studies in progress on the chemical purification of CRF may yield more definitive data on the problem of its relation to prothrombin.

Summary

A comparative study of the action of dicumarol and of phenylindanedione on the coagulase reacting factor and on prothrombin is presented.

Intramuscular administration of dicumarol and of PID in rabbits reduces the plasma levels of CRF as well as of prothrombin.

The response of CRF and of prothrombin to the anticoagulants does not consistently proceed *pari passu*.

Vitamin K_1 (mephyton) successfully reverses the action of both anticoagulants on CRF and on prothrombin.

The implications of these observations on the problem of the relationship of CRF to prothrombin are discussed.

Acknowledgments

The author is indebted to Miss Grace Ullman and Mrs. Edith Voigt for technical assistance and to Dr. E. A. Sharp and Dr. J. E. Gajewski of Parke, Davis and Company for making phenylindanedione available.

References

1 Alexander, B., De Vries, A., and Goldstein, R.: Prothrombin: a critique of methods for its determination and their clinical significance. New Eng. J. M., 1949, *240*, 403.

2 Alexander, B., Goldstein, R., and Landwehr, G.: The prothrombin conversion accelerator of serum (SPCA), its partial purification and its properties compared with serum Ac-globulin. J. Clin. Invest., 1950, *29*, 881.

3 Duthie, E. S. and Lorenz, L. L.: Staphylococcal coagulase: mode of action and antigenicity. J. Gen. Microb., Lond., 1952, *6*, 95.

4 Jaques, L. B., Gordon, E., and Lepp, E.: A new prothrombopenic drug, phenylindanedione. Canadian M. Ass. J., 1950, *62*, 465.

5 Meunier, P., Mentzer, C., and Molhe, D.: Antivitamin K hemorrhagic activity of an indanedione. C. rend. Acad. sc., 1947, *29*, 977.

6 Miale, J. B.: The rôle of coagulase-globulin in blood coagulation and its thromboplastic action. Am. J. Clin. Path., 1952, *22*, 218.

7 Owen, C. A. and Bollman, J. L.: Prothrombin conversion factor of dicumarol plasma. Proc. Soc. Exp. Biol., N. Y., 1948, *67*, 231.

8 Smith, W. and Hale, J. H.: The nature and mode of action of staphylococcus coagulase. Brit. J. Exp. Path., 1944, *25*, 101.

9 Soulier, J. and Gueguen, J.: Action hypoprothrombinemiante (anti K) de la phénylindane-dione étudiée experimentalement chez le lapin. Son application chez l'homme. C. rend. Soc. biol., 1947, *141*, 1007.

10 Tager, M. and Hales, H. B.: Studies on the coagulase-reacting factor. II. Properties of coagulase-reacting factor, and relation to blood clotting components. J. Immun., Balt., 1948, *60*, 1.

11 Tager, M. and Lodge, A. L.: Influence of the physiological blood clotting process on the coagulation of blood by staphylocoagulase. J. Exp. M., 1951, *94*, 73.

12 Tager, M. and Lodge, A. L.: Changes in the properties of the coagulase-reacting factor of plasma after separation from prothrombin by Seitz filtration. J. Immun., Balt., 1951, *67*, 63.

13 Ware, A. G. and Stragnell, R.: An improved one-stage prothrombin method. Am. J. Clin. Path., 1952, *22*, 791.

A MECHANISM OF REGULATION OF FOOD INTAKE*

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Regulation of food intake is a term which includes all of the reactions through which an animal (i) maintains a constant intake while environmental and metabolic conditions are constant, and (ii) adjusts its intake whenever environmental or metabolic conditions are altered. What an animal eats and how much are usually thought to be determined by the energy requirements of the body; but every careful observer who has conducted feeding experiments knows that there are many circumstances in which the amount of food eaten is not related to energy expenditure. For example, most mammals will not eat when they are deprived of water, because the demand for energy is secondary to preventing dehydration. They also fail to eat when placed in a hot environment with free access to water and food. There is, therefore, no simple correlation between energy needs and food intake which is valid under all conditions, and it is our purpose now to present the hypothesis that the important factor in regulation of food intake is not its energy value, but rather the amount of extra heat released in its assimilation. Our data do not exclude the possibility that there are other bases for this regulation; as there are several factors in regulation of respiration (CO_2 tension, pH, O_2 tension, etc.), so there may be more than one factor in regulation of feeding. Yet the intake of energy as food seems to a large extent to be regulated indirectly via the heat liberated in assimilating food, much as oxygen intake is regulated via carbon dioxide concentration and pH of the blood.

Other theories of hunger, appetite, and satiety, based on Cannon's[9] and Carlson's[10] studies of sensation from the stomach, do not account in a quantitative way for regulation feeding, nor explain the observation that animals eat different amounts of different diets. The ability of animals to change their intake when the composition of food is altered suggests that animals recognize some quality in food, and that this quality may be correlated with regulation of the amount of food eaten. This quality we have arbitrarily called the intrinsic food factor, and we have concluded that it may be identical with the specific dynamic action, since it cannot be any one of the more obvious dietary constituents, including the caloric value of the food. Following feeding, the heat production is greater than before the food

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Received for publication March 12, 1953.

was taken. This extra heat, called the "specific dynamic action" by earlier physiologists (Rubner), must be disposed of in some way, along with the calories, protein, carbohydrate, fat, vitamins, and minerals of the food. As shown by the data of Strang and McClugage,[31] this increase in heat production is rather abrupt, especially when the rate of change of heat production is plotted graphically against time. Booth and Strang[4] further noted in human subjects that the S.D.A. of food brings about a postprandial rise in skin temperature. By measuring the rate of this rise and correlating it with the amount of food eaten by thin, by normal, and by obese subjects, they concluded that warming of the skin is an essential part of satiety reactions. Rony[27] later criticized their concept on the grounds that the quantity of heat produced was too small to warm the body by the amount that Booth and Strang had noted, but it appears that Rony failed to consider the possibility that the skin becomes warmer, not because it is directly warmed by the extra heat, but because in response to the elevated heat production there is an active cutaneous vasodilatation.

The S.D.A. is known to vary with the metabolic state of the animal[8, 21] which is determined, in part, by the metabolic mixture which the animal consumes.[19, 20, 28] During adjustment to a new diet the activity of various enzyme systems changes,[20, 28] and there may be at the same time, perhaps associated with alterations in metabolic pathways, some modification in the amount of heat liberated from food. It is important to recall that the S.D.A. of a diet is not the same as the sum of the S.D.A.'s of the individual constituents of the diet, but depends, rather, upon the relative proportions of protein, carbohydrate, and fat.[14, 16] Thus, the specific dynamic action of protein, alone, is different from its S.D.A. when fed in a mixture with other foodstuffs. If the S.D.A. is the intrinsic food factor which the animal takes in constant amount from day to day via its food, a change in the magnitude of the S.D.A. of any component of the diet would result in a corresponding change in the amount of food eaten. Changes of this type would occur gradually in company with the gradual metabolic changes, and therefore a latent period of some days would intervene before an animal previously stabilized on one diet would make a final adjustment to a new food mixture. In our experiments where fat, carbohydrate, or protein was added to a mixed diet, such gradual changes in food intake did occur.[22] The nature of these adjustments was as follows: On the first day following the change, the intake (in grams or calories) might be quite different from the animal's previous intake, but when the number of S.D.A. calories was calculated (using published data for the S.D.A.) for the control period and for the first day following the change in diet, values for intake were obtained which were more nearly constant. On the succeeding 3-8 days, gradual changes occurred in intake during the interval when changes in metabolism would be expected to result from the altered metabolic mixture. Our data suggest that the reason that the caloric intake is mistakenly believed to be the

factor regulated by animals is that on a diet of constant composition the caloric value and S.D.A. are always proportional; consequently, in the absence of any metabolic change it may be impossible to decide which of the two factors is being maintained at a constant level.

As an example of the type of calculation that can be made in studying this regulation, Lusk's figures for S.D.A. (i.e., 30% for protein, 4.9% for carbohydrate, and 4.1% for fat)[21] have been applied to the food intake of normal rats receiving a diet made up of a mixture of natural foods (G.L.F. calf meal), and subsequently diluted with various proportions of fat.[22] When the percentage of added fat was increased from zero to 14, 43, and 57% by weight, the total caloric intake per day more than doubled on the first day;

TABLE 1

SPECIFIC DYNAMIC ACTION OF FOOD EATEN IN 18 HOURS WHEN LARD WAS ADDED TO DIET

May 2, 1946—Sprague-Dawley Males (300-325 gm.)

% Lard added to diet	*Total caloric intake*	*Partition of caloric intake*			*Calculated SDA, kcal.*			*Total SDA, kcal.*
		Protein	*CH_2O*	*Fat*	*Protein*	*CH_2O*	*Fat*	
0	54.8	13.2	36.7	4.9	4.0	1.8	0.2	6.0
14	66.4	11.0	30.9	24.5	3.3	1.5	1.0	5.8
43	90.0	7.2	19.8	63.0	2.2	1.0	2.6	5.8
57	116.3	5.8	17.5	93.0	1.7	0.9	3.8	6.4
86	110.0	1.6	4.4	104.0	0.5	0.2	4.3	5.0
100	104.6	0.0	0.0	104.6	0.0	0.0	4.3	4.3

but the total estimated S.D.A. remained nearly constant at 6.0, 5.8, 5.8, and 6.4 kcal./day, respectively (Table 1).[22] Using data for S.D.A. obtained from other sources, however, the results of certain of our experiments are less uniform, and some of our results do not yield constant amounts of extra heat per day even when Lusk's figures are used in the calculation. In spite of the many experiments performed by other investigators measuring the S.D.A., we have not been able to determine with certainty from published data what the S.D.A. may be when both the composition of the diet and the metabolic state are varying. It appears that in order to test the hypothesis further, measurements of the S.D.A. of the mixed diets will have to be done simultaneously with the experiments in which food intake is measured. But in spite of this limitation of our knowledge, of all of the variables which we measured, the estimated S.D.A. was the most nearly constant on the first day of changed dietary composition—much more nearly constant than factors such as caloric intake, total weight of food, bulk of food eaten, or the intake of either protein, carbohydrate, or fat.

Energy obtained from food is utilized by animals to do work, to increase bodily stores of carbohydrate, protein, and fat, and to maintain body temperature—all of which are accompanied by the loss from the body of rather considerable amounts of heat. Since heat production is common to all of these avenues of expenditure, it is reasonable to suppose that their regulation must be related in some manner to the body's temperature regulation, and that they must undergo some integration so that no one of these factors is allowed to vary independently of the others. An example of the interdependence existing among these reactions may be noted in female rats, where cycles of body temperature, body weight, food intake, and spontaneous activity (running) are correlated with the estrous cycle.[7] Similarly, in male rats, food intake is altered by changes in environmental temperature,[8] as it is also in other species, including cattle (Brody)[5] and human infants (Cooke).[11] All of these variations appear consistent with the idea that food intake is regulated by way of the extra heat produced in its assimilation, in that the intake decreases when the animal is having difficulty in losing heat in a warm environment, or when heat production rises because of increased activity at a constant temperature.

The experiments of Grossman, Ivy, and their associates,[15, 17, 29] the data of Anand and Brobeck,[3] and our own results all indicate that the regulation of food intake is accomplished by the central nervous system, rather than by the stomach (Carlson)[10] or by the stomach and duodenum (Alvarez).[2] In brief, the nervous system apparently serves for the integration of a number of mechanisms having the following functions:

1. Motor activity, especially locomotion;
2. Feeding reflexes;
3. Facilitation of feeding reflexes, and of locomotion;
4. Inhibition of feeding reflexes, and of locomotion;
5. Recognition of food.

Of these, the term "feeding reflexes" is generally used and probably does not need further definition, except to say that it should be applied only to a reaction initiated by some definite sensory stimulation. The stimulus may be visual, olfactory, auditory, tactile, or through taste, and may serve to direct the organism to an object, may lead to "mouthing" or oral investigation, to chewing and swallowing. If the object is not appropriate for food, a normal animal will reject it somewhere along this series of reactions, whereas an animal deprived of portions of the cerebral cortex, particularly the "visceral" cortex, will not reject it but will eat it as though it were food (Pribram).[28] This observation is responsible for the impression that in higher animals the recognition of food is primarily a cortical function.

The nervous system evidently drives, impels, or urges the individual to eat; this drive becomes apparent as restlessness, locomotion, searching, or "rooting," depending upon the species and age of the animal. From this drive arises the hyperactivity which Sherrington[30] and, more recently,

Wald[34] interpreted as a useful reaction, increasing the likelihood that food will be encountered. A satiated animal under natural conditions is not likely to discover more food because it fails to move about; hence, the urge to locomotion is an essential preliminary to feeding. In human infants, restlessness, irritability, and crying serve the same purpose, since the mother performs the movements necessary to bring the baby near to the source of food. If it were not for the storage capacity of the gut, eating probably would not be periodic, and the "normal" or habitual waking state would be a roving one, a moving from place to place, investigating, mouthing, and feeding whenever possible.

Hetherington's[18] observation (which we have confirmed) that rats with hypothalamic lesions often show reduced locomotor activity, as well as Miller, Bailey, and Stevenson's[24] discovery that similar animals, although they may exhibit hyperphagia, are unwilling to work for their food, together point to the conclusion that at least certain portions of the hypothalamus take part in maintaining the urge to locomotion and motor activity. On the other hand, the complete aphagia in the presence of food noted by Anand and Brobeck[3] in rats and cats with lesions in the lateral hypothalamus suggests that the hypothalamus also has some more specific function in feeding, a function which is most conveniently described as facilitation of feeding reflexes. Magoun[22] has shown that the hypothalamus contains an important part of the "bulbar facilitatory mechanism," a neural system capable of determining whether or not other parts of the nervous system can become active, as well as their level of activity. An afferent stimulus too weak to excite a reflex action may produce a response if the facilitatory mechanism is excited simultaneously. In the hypothalamus the facilitatory mechanism occupies the same lateral location as the "feeding center" of Anand and Brobeck—a localization suggesting that feeding reflexes may require central facilitation. The sensations produced by hunger contractions may have similar effects, viz., increasing motor activity and facilitating feeding reflexes, since both Sherrington and Carlson noted that "hunger" is associated with generalized hyperexcitability of the central nervous system.

Another function of the hypothalamus may be the inhibition of eating. When lesions are placed in the medial hypothalamus, animals become hyperphagic and obese[5] even though their "drive" or "urge" to eat has been reduced. The hyperphagia may be a result of destruction of cells normally responsible for the inhibition of feeding. It is conceivable that they inhibit either the facilitatory portion of the lateral hypothalamus, or possibly the feeding reflexes directly. Whether stimuli from the gastro-intestinal tract in normal animals act on the hypothalamus or at the reflex level is unknown. Whereas hunger contractions appear to increase activity and to facilitate feeding reflexes, distention of the stomach and intestine seems to inhibit both motor output and reflex activity. Yet Grossman's[15] results using animals with denervated gastro-intestinal tracts show that feeding can proceed

normally without sensation from stomach and intestine, presumably because the central mechanisms are affected by factors other than visceral stimulation. Sherrington[30] and Pavlov[25] suggested some years ago that feeding may be regulated in a manner analogous to respiration, where the excitatory state of the respiratory center is modified by the composition of the blood, by impulses arising peripherally in the lungs, and by activity in other portions of the neural axis. In terms of the hypothesis which we propose, the specific dynamic action of food would play in the regulation of food intake a rôle analogous to the chemical changes which act centrally to modify respiratory exchange. Any increase in the total amount of heat in the body, acting through thermal gradients and thermal receptors, would initiate impulses inhibiting the mechanisms responsible for feeding. An increase in environmental temperature would do the same. When environmental temperature decreased, or when heat production diminished, a reversal of the thermal gradients would remove the inhibition so that the animal would once again become active, searching for food and ready to eat. The hypothalamic mechanisms conceivably might be sensitive to temperature changes in the blood, as Magoun and his associates[35] demonstrated in their studies of the responses following direct heating of the hypothalamus. Data now available do not exclude the possibility, however, that some other reaction may be interposed between the released heat and the neural activity—possibly some movement of water or electrolytes, some change in cellular permeability, or in the rate of certain chemical reactions within the sensitive cells. Verney[33] has proposed a concept similar to this to explain his results with perfusion of the hypothalamic region in dogs. He suggested that alterations in the osmotic pressure of the carotid blood are adequate stimuli for the supraoptico-hypophysial complex, via osmoreceptors whose rate of discharge is affected by gradients of water concentration. He mentioned no observations upon thermoregulation of his animals during the experiments, although there is other evidence that water balance, food intake, and regulation of body temperature are interdependent.[12] Darrow and Yannet[18] found that animals subjected to depletion of their extracellular electrolyte (following injection intraperitoneally of isotonic glucose solution) failed to eat and drink, while Cort[13] observed aphagia following subcutaneous injection of small amounts of hypertonic saline. Whether this failure to eat was caused directly by shifts of body water and electrolyte is unknown, and these experiments are mentioned here only to emphasize the point that generalized changes in composition of body fluids are capable of affecting the activity of mechanisms regulating energy exchanges between animals and their environment.

Summarizing our hypothesis, we suggest that a normal animal when "hungry" is driven by his central nervous system into extra locomotion and searching, while his feeding reflexes are brought into a facilitated state. Following feeding, the locomotion decreases and the reflexes are no longer facilitated, in fact, they may be actively inhibited. Stimuli reaching the

central nervous system from the gut apparently modify both the level of motor output and the state of feeding reflexes. But factors acting upon other parts of the body, possibly directly upon the central nervous system, are equally important in feeding behavior. One such factor is represented by the conditions of temperature regulation which, through the specific dynamic action (S.D.A.) of food and by way of environmental temperature, appear to have important effects upon food intake. Other factors undoubtedly exist, with the result that the central regulation of feeding is accomplished through integration of a variety of types of disturbances and reactions within the organism or between it and its environment.

References

1 Adolph, E. F.: Urges to eat and drink in rats. Am. J. Physiol., 1947, *151,* 110.

2 Alvarez, W. C.: *An introduction to gastro-enterology.* New York, Hoeber, 1940, 778 pp.

3 Anand, B. K. and Brobeck, J. R.: Hypothalamic control of food intake in rats and cats. Yale J. Biol., 1951, *24,* 123.

4 Booth, G. and Strang, J. M.: Changes in temperature of the skin following the ingestion of food. Arch. Int. M., 1936, *57,* 533.

5 Brobeck, J. R.: Mechanism of the development of obesity in animals with hypothalamic lesions. Physiol. Rev., 1946, *26,* 541.

6 Brobeck, J. R.: Effects of variations in activity, food intake and environmental temperature on weight gain in the albino rat. Am. J. Physiol., 1945, *143,* 1.

7 Brobeck, J. R., Wheatland, Mary, and Strominger, J. L.: Variations in regulation of energy exchange associated with estrus, diestrus and pseudopregnancy in rats. Endocrinology, 1947, *40,* 65.

8 Brody, S.: *Bioenergetics and growth.* New York, Reinhold Publishing Corp., 1945, 1023 pp.

9 Cannon, W. B. and Washburn, A. L.: An explanation of hunger. Am. J. Physiol., 1912, *29,* 441.

10 Carlson, A. J.: *Control of hunger in health and disease.* Chicago, Ill., University of Chicago Press, 1916, 2d impression, 319 pp.

11 Cooke, R. E.: Behavioural response of infants to heat stress. Yale J. Biol., 1952, *24,* 334.

12 Cort, Ruth L.: Interrelationship of hunger and thirst. Yale University, School of Medicine, Thesis submitted 1951.

13 Darrow, D. C. and Yannet, H.: Metabolic studies of the changes in body electrolyte and distribution of body water induced experimentally by deficit of extracellular electrolyte. J. Clin. Invest., 1936, *15,* 419.

14 Forbes, E. B. and Swift, R. W.: Associative dynamic effects of protein, carbohydrate and fat. J. Nutrit., 1944, *27,* 453.

15 Grossman, M. I., Cummins, G. M., and Ivy, A. C.: The effect of insulin on food intake after vagotomy and sympathectomy. Am. J. Physiol., 1947, *149,* 100.

16 Hamilton, T. S.: The heat increments of diets balanced and unbalanced with respect to protein. J. Nutrit., 1939, *17,* 583.

17 Harris, S. C., Ivy, A. C., and Stearle, Laureen M.: The mechanism of amphetamine-induced loss of weight. J. Am. M. Ass., 1947, *134,* 1468.

18 Hetherington, A. W. and Ranson, S. W.: The spontaneous activity and food intake of rats with hypothalamic lesions. Am. J. Physiol., 1942, *136,* 609.

19 Lundbaek, K. and Stevenson, J. A. F.: Reduced carbohydrate intake after fat feeding in normal rats and rats with hypothalamic hyperphagia. Am. J. Physiol., 1947, *151,* 530.

20 Lundbaek, K. and Stevenson, J. A. F.: The effect of previous carbohydrate deprivation on the carbohydrate metabolism of isolated muscle. Fed. Proc., Balt., 1948, *7,* 75.

21 Lusk, G.: *The elements of the science of nutrition.* Philadelphia, W. B. Saunders Co., 1928, 4th ed., Chapt. XII, pp. 276-308.

22 Magoun, H. W.: Caudal and cephalic influences of the brain stem reticular formation. Physiol. Rev., 1950, *30,* 459.

23 Magoun, H. W., Harrison, F., Brobeck, J. R., and Ranson, S. W.: Activation of heat loss mechanisms by local heating of the brain. J. Neurophysiol., 1938, *1,* 101.

24 Miller, N. E., Bailey, C. J., and Stevenson, J. A. F.: Decreased "hunger" but increased food intake resulting from hypothalamic lesions. Science, 1950, *112,* 256.

25 Pavlov, I. P.: *Lectures on conditioned reflexes.* New York, International Publishers, 1928, 414 pp.

26 Pribram, K. H.: Cited in Fulton, J. F.: *Frontal lobotomy and affective behavior.* New York, W. W. Norton & Co., 1951, pp. 78-79.

27 Rony, H. R.: *Obesity and leanness.* Philadelphia, Lea and Febiger, 1940.

28 Samuels, L. T.: Body adaptation to change in diet. J. Am. Diet. Ass., 1946, *22,* 843.

29 Sangster, W., Grossman, M. I., and Ivy, A. C.: Effect of d-amphetamine on gastric hunger contractions and food intake in the dog. Am. J. Physiol., 1948, *153,* 259.

30 Sherrington, Sir Charles: Cutaneous sensation, in: *Textbook of Physiology,* (Sharpey-) Schaefer, E. A., Ed., Edinburgh, Pentland, Vol. 2, 1900, pp. 920-1001.

31 Strang, J. M. and McClugage, H. B.: The specific dynamic action of food in abnormal states of nutrition. Am. J. M. Sc., 1931, *182,* 49.

32 Strominger, J. L., Brobeck, J. R., and Cort, R. L.: Regulation of food intake in normal rats and in rats with hypothalamic hyperphagia. Yale J. Biol. (To be published)

33 Verney, E. B.: Absorption and excretion of water. Lancet, Lond., 1946, *251,* 739.

34 Wald, G. and Jackson, Blanche: Activity and nutritional deprivation. Proc. Nat. Acad. Sc., U. S., 1944, *30,* 255.

ADAPTATION OF INFLUENZA VIRUS TO MICE. I. GENETIC AND ENVIRONMENTAL FACTORS AFFECTING AN A PRIME STRAIN OF INFLUENZA VIRUS*†

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Although a number of important studies have recently unravelled some of the mystery associated with the process of adaptation of influenza virus to mice and hamsters, little is known concerning the factors which are operating to promote or to delay the acquisition of pathogenicity.§[2-6, 7, 11-14] After four or five serial passages in mice, most Type A strains become lethal, causing extensive pulmonary consolidation.[5] Most of the data on Type A prime strains consist of a comparison of the growth curves of mouse-adapted and mouse-unadapted lines.[5, 10, 14] In general Type B strains of influenza virus have proven difficult to adapt to mice, although the addition of certain adjuvants of bacterial and non-bacterial origin to the virus preparations appears to promote the acquisition of pathogenicity.[7] To date, no comparable studies on Type C strains have been published.

The SEEREY Type A prime strain was found to multiply readily in murine lungs in the majority of instances. Even after more than 15 serial passages in mice, however, it failed to become lethal or to cause extensive pulmonary consolidation. In view of these properties the SEEREY strain was selected for more extensive studies designed to ascertain the factors which might operate to hasten or to retard the process of adaptation. The present paper is concerned with the influence of the genetic background of the mice, and the environmental temperature at which they are kept, on the development of the pathogenic property of the virus.

MATERIALS AND METHODS

Virus. The SEEREY strain of Type A prime influenza virus was obtained from Dr. Robert H. Green who isolated it in New Haven in 1947. A mouse-passage line (SEEREY E6M11-12) passaged 6 times in eggs and 11 or 12 times in mice, and an egg line (SEEREY E9-10) passaged 9 or 10 times in eggs, were employed. The mouse passages occurred in a heterogenetic strain of albino mice which had been maintained

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† This study was supported by a contract between the Office of Naval Research and Hahnemann Medical College, and in part by a grant from the Sidney Hillman Medical Center Research Fund.

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§ By adaptation is meant the process whereby the virus acquires the ability to produce lesions and death in mice on serial passage. The term is used synonymously with the acquisition of pathogenicity. It is to be distinguished from the capacity of some strains of the virus when inoculated in high concentration to produce a fatal pneumonia in mice which cannot be passed serially.

Received for publication March 6, 1953.

in the Department of Microbiology at Yale University for a number of years. These mouse and egg lines produced neither pulmonary consolidation nor death in mice at the time the investigation was initiated. However, important differences in the behavior of the mouse and the egg lines were noted in the studies herein reported.

The Melbourne (MEL-M) strain of Type A influenza virus was obtained from Dr. F. M. Burnet who isolated it in 1935. It had numerous passages in mice. Suspensions of mouse lungs infected with the virus were lethal in dilutions of 10^{-4}.

Virus suspensions used for passage. The lungs of infected mice were removed four days after inoculation and ground in a mortar with sterile alundum. Lung suspensions were prepared by the addition of 1 ml. of saline per set of lungs. The lung suspensions were centrifuged at 1500 rpm. for five minutes and the resulting supernates, which represented the stock suspensions of the viruses, were considered to be undiluted. On a weight basis this would be approximately a 20 per cent suspension. Infected allantoic fluids were harvested 44 hours after inoculation and diluted ten-fold in saline. In general, virus suspensions were used immediately after they were prepared. In several instances they were stored under dry ice in a tightly stoppered test tube at temperatures varying from —50° to —60° C. During the course of the investigation it became apparent that, unlike previous experience with lung suspensions of Type A influenza virus, under these conditions of storage there was frequently a drop in egg infectivity. For this reason, in the recording of the data in the tables, the lung suspensions which were stored in dry ice before they were tested for egg infectivity have been so designated.

Titrations of egg infectivity. Serial ten-fold dilutions of virus suspensions were made and a volume of 0.05 ml. of the desired dilution of virus was inoculated into the allantoic cavity of fertile chicken eggs which had been incubated at 38° C. for 10 or 11 days prior to inoculation. Four or six eggs were used for each dilution. The inoculated eggs were incubated at 35° C. for 44 hours before harvest. Allantoic fluid from each egg was tested for agglutination of chicken erythrocytes by a pattern method[10] and the 50 per cent infectivity titer (EID_{50}) was calculated.[8]

Mice. NIH (National Institute of Health) albino mice were obtained from a local dealer. They are heterogenetic and have not been inbred. CF1 (Carworth Farms albino with brown non-agouti background) and CFW (Carworth Farms albino agouti) mice were obtained from Carworth Farms. They have been inbred for several generations. All mice were used when they weighed approximately 18 to 22 grams.

Serial passage in mice. A volume of 0.05 ml. of virus suspension was inoculated into groups of three or six lightly anesthetized mice. After four days the mice were sacrificed, the lungs examined for lesions which were scored according to the method of Horsfall.[6] The lungs were then used to prepare a suspension of virus for passage. A 1 : 10 dilution of the lung suspension was used for serial passage. At periodic intervals during the course of serial passage of the virus the EID_{50} of the inoculum was determined. In addition mice inoculated with the lung suspensions were frequently observed for 10 days after inoculation for pulmonary lesions and death.

Temperature. For certain experiments a temperature of 5° C. was maintained by placing the mice in a low temperature incubator. If sufficient aeration of the low temperature incubator was provided by means of a Model A Marco Air Pump (manufactured by the J. B. Maris Company of Bloomfield, New Jersey), uninoculated mice appeared to suffer no ill effects from the low temperature. No marked alterations in bacterial flora of the respiratory tract were observed. Normally mice were kept at temperatures between 19° and 22° C. in an air-conditioned room.

Receptor destroying enzyme (RDE). Preparations of RDE were obtained from the 4 Z strain of *V. cholerae* by the inoculation of the allantoic cavity of 12-day chick

embryos.[1] RDE was employed in amounts of approximately 0.1 unit titrated according to the method of Burnet and Stone.[1] It was contained in a volume of 0.05 ml. and injected intranasally under light anesthesia.

Experimental Results

On initial inoculation of a heterogenetic strain of albino mice (Yale University) with infected allantoic fluid containing high concentrations of the SEEREY strain of influenza virus, pulmonary lesions were produced. In five out of eight experiments virus persisted for 10 or more serial passages

Table 1

Comparison of Adapted and Unadapted Strains of Influenza Virus in Mice of Different Genetic Constitution

Virus	*Inoculum log EID_{50}*	*Mice*	*% Pulmonary consol.*†	*Mortality*‡
SEEREY (M)	4.2	NIH	6	0/12
unadapted		CF1	20	0/12
		CFW	35	0/12
MEL-M	6.7	NIH	20	0/12
adapted		CF1	50	1/12
		CFW	83	5/12

EID_{50}—the 50 per cent infectivity titer for eggs. (M)—mouse line.
† The extent of consolidation was recorded four days after inoculation of the virus.
‡ The number of mice which died in four days over the number inoculated. All deaths in this particular experiment occurred on the fourth day after the inoculation of virus.

as determined by the infectivity of the mouse lungs for eggs. However, it proved impossible to pass the lesions in series. Various adjuvants including the daily intraperitoneal injection of cortisone and the intranasal instillation of saline failed to promote adaptation of the virus to mice although the virus persisted through 15 serial passages. These preliminary studies indicated that serial pasage in mice at four-day intervals was more likely to permit the continued multiplication of the virus than passage at three-day intervals. It was also observed that serial passage employing a 10^{-1} dilution of the lung suspension was preferable to passage in a 10^{-2} dilution.

The data recorded in Table 1 suggested that the strain of mice which was used for serial passage might prove to be an important factor in the process of adaptation. Four days after inoculation of the SEEREY strain pulmonary lesions were more marked in CF1 and CFW mice than in NIH mice. When the pathogenic MEL-M strain was inoculated, comparable differences were observed, and in addition the lethal effect was more marked in the CFW mice. Furthermore, the 50 per cent egg infectivity (EID_{50}) of the mouse lungs infected with the SEEREY strain was more than 1 log higher

in the CFW mice than in the NIH or CF1 mice. On the basis of these results serial passage of the SEEREY strain in CFW and CF1 mice was undertaken.

From the results included in Table 2 it is apparent that during the course of serial passage in CFW mice the SEEREY virus had adapted, as determined

TABLE 2

ADAPTATION OF THE SEEREY STRAIN OF INFLUENZA VIRUS IN CFW MICE

	% Pulmonary consol.†		*log MS_{50}*		*log EID_{50}***	
Passage§	*CFW*	*CF1*	*CFW*	*CF1*	*CFW*	*CF1*
0	40	25				
1	16	16			4.0	2.8
2	10	10				
3	0	0				
4	10	5				
5	10	10	>1.0	1.0		
6	25	5				
7	40	5				
8	60*	5				
9	20	5			3.5	3.5
10	15	15	1.0	<1.0		
11	60*	20				
12	75	15				
13	75*	10			5.5	3.6
14	90	15				
15	25	25	>1.0	<1.0	5.3	2.7
16	30		>3.0‡		4.7	
18	80*		2.8‡		4.9	
27	70*		3.0‡			

MS_{50}—the 50 per cent maximum score of the lesions.
EID_{50}—the 50 per cent infectivity titer for eggs.

† The extent of consolidation was recorded on the fourth day following inoculation of virus at the time when serial passage was made.

* Deaths occurred in some or all of the mice in the group.

** These preparations were stored in dry ice before infectivity titrations were carried out in eggs.

‡ The log of the 50 per cent lethal dose (LD_{50}) was about 1 log lower.

§ This virus previously had 12 serial mouse passages.

by the ability of the virus to produce pulmonary lesions which may be passed in series and by its lethal effect which may be passed serially. After the fifteenth passage in CFW mice the MS_{50} of the virus was 10^{-3}, i.e., 50 per cent of the pulmonary area was consolidated in mice inoculated with lung suspensions which were diluted a thousand-fold. Although some mice occasionally died when inoculated with a 10^{-3} dilution of the mouse lungs, the dose that killed 50 per cent of the mice (LD_{50}) was usually about 10^{-2} to

$10^{-2.7}$. It should be noted that the SEEREY virus used to initiate these serial passages was the mouse-passaged line which had twelve passages in Yale University mice. Unfortunately, the supply of CF1 mice was exhausted after fifteen serial passages and could not be replenished for several weeks.

TABLE 3

ADAPTATION OF THE SEEREY STRAIN OF INFLUENZA VIRUS IN CFW MICE KEPT AT DIFFERENT TEMPERATURES AFTER INOCULATION

	% Pulmonary consol.†		*Mortality‡ in 4 days*		*Mortality‡* in 10 days*		*log EID_{50}***	
Passage§	*5° C.*	*RT*	*5° C.*	*RT*	*5° C.*	*RT*	*5° C.*	*RT*
0	20	50	0/3	0/3				
1	10	20	0/3	0/3			5.3	5.7
2	30	30	0/3	0/3				
3	25	40	0/3	0/3				
4	40	25	3/3	0/3	8/12	3/12	6.5	4.5
5	50	60	0/3	0/3				
6	75	75	1/3	0/3				
7	85	75	0/3	0/3				
8	85	50	1/3	0/3				
9	75	75	3/3	1/3	6/6	5/6	6.5	6.0
10	75	45	2/3	0/3				
11	70	40	1/3	0/3				
12	90	70	3/3	1/3				
13	45	20	1/3	0/3				
14	90	80	3/3	2/3	4/6	5/6	7.3	5.7
15	85	60	3/3	1/3				
15	85	60	3/3	1/3				
20	90	75	3/3	1/3				
23	70	70	3/3	2/3				
24	90	75	3/4	2/3	5/6	5/6	5.5	5.5

EID_{50}—the 50 per cent infectivity titer for eggs.
RT—room temperature which varied between 19° and 22° C.
† The extent of consolidation was recorded on the fourth day following inoculation of virus at the time when serial passage of the mouse lungs was made.
‡ The number of mice which died over the number inoculated.
* These mice were kept at room temperature—between 19° and 22° C.
** The 50 per cent infectivity titers were determined on the mouse lungs obtained four days after inoculation of virus.
§ This virus previously had 12 serial mouse passages.

Although the virus persisted in the lungs of CF1 mice through 15 serial passages, adaptation had not yet occurred. However, when the serial passages were later continued in CF1 mice, it became apparent that the virus had adapted in these mice after three or four additional passages. The data indicate that in this particular instance at least adaptation occurred more rapidly in CFW mice than in CF1 mice.

When the effect of environmental temperature on the process of adaptation of the SEEREY strain of influenza virus in CFW mice was examined, the results suggested that temperatures of 5° C. slightly enhanced the process of adaptation. From the data included in Table 3, however, it is apparent that adaptation was also occurring in the mice kept at room temperature. It appeared probable that if temperatures of 5° C. actually did exert a significant effect on the process of adaptation of the virus, an experiment employing the most resistant NIH mice might prove more fruitful. Before commencing with the serial passages in the NIH mice, a few preliminary experiments were undertaken to ascertain if the egg infectivity of the mouse lungs would be higher in mice kept at 5° C. as opposed to those maintained at room temperature. The results recorded in Table 4 show that

TABLE 4

EFFECT OF TEMPERATURE AND GENETIC BACKGROUND ON MULTIPLICATION OF THE SEEREY STRAIN OF INFLUENZA VIRUS IN MICE

Allantoic Fluid	*Inoculum log EID_{50}*	*Temp.*	*log EID_{50} of mouse lungs 4 days after inoculation*		
			NIH	*CF1*	*CFW*
SEEREY (E)	5.5	5 C	4.5*	2.9*	4.2*
		RT	<1.0*	2.0*	3.6*
SEEREY (E)	7.3	RT	2.7*		6.3*
SEEREY (M)	4.5	5 C	6.3		
		RT	4.3		
SEEREY (M)	4.2	RT		2.8*	4.0*

EID_{50}—the 50 per cent infectivity titer for eggs.
RT—room temperature which varied between 19° and 22° C.
* These preparations were stored in dry ice before infectivity titrations were carried out in eggs.
(E)—egg line. (M)—mouse line.

if NIH mice are kept at 5° C., appreciably higher concentrations of virus are present in the mouse lungs four days after inoculation when contrasted with NIH mice at room temperature. This finding was observed both with the egg-passaged and the mouse-passaged line of the virus. The significance of the higher titers of virus in the NIH mice maintained at 5° C. is not known, but these higher concentrations of virus might be important in the serial passage of the infected mouse lungs and in the process of adaptation of the virus. In contrast there were no similarly marked differences in the virus concentrations in CFW mice kept at the different temperatures. It is apparent from the tabulated data that there is an extremely marked difference in the levels of virus multiplication in the NIH mice as contrasted with the CFW mice. The genetic background of the mouse certainly exerts a

marked effect on the multiplication of the SEEREY virus. An excellent example of the effect of the genetic background of the host on virus multiplication has been reported recently by Sabin[9] in his studies of the resistance of mice to the intracerebral injection of the 17 D strain of yellow fever virus.

The tabulated results indicate that when the serial passages of the mouse line of the SEEREY virus are carried out in NIH mice, extensive pulmonary consolidation is produced (Table 5). The process of adaptation is observed to occur more rapidly in the NIH mice maintained at 5° C. than in the

TABLE 5

THE EFFECT OF TEMPERATURE ON THE SERIAL PASSAGE OF THE SEEREY STRAIN OF INFLUENZA VIRUS IN NIH MICE

	% Pulmonary consol.†		*Mortality*‡		*log EID_{50}*	
Passage§	*5° C.*	*RT*	*5° C.*	*RT*	*5° C.*	*RT*
0	30	30	0/3	0/3		
1	30	10	0/3	0/3	6.3	4.3
2	45	15	3/3	0/3		
3	30	10	0/3	0/3		
4	70	5	1/3	0/3	6.7	4.5
5	50	0	1/3	0/3		
6	40	10	0/3	0/3		
7	30	15	3/3	0/3		
9	60	70	3/3	1/3	6.8	4.5

EID_{50}—the 50 per cent infectivity titer for eggs.
RT—room temperature which varied between 19° and 22° C.
† The extent of consolidation was recorded on the fourth day following inoculation of virus when serial passage of the mouse lungs was made.
‡ The number of mice which died in four days over the number of mice inoculated.
§ This virus previously had 12 serial mouse passages.

NIH mice at room temperature. An interesting difference between the mice at 5° C. and those at room temperature is concerned with the significantly higher concentrations of virus which are present in the lungs of the NIH mice at 5° C. It should be noted that the infected mouse lungs were assayed for virus on the fourth day after inoculation. Current experiments dealing with the "growth curves" of the virus in mice at 5° C. and at room temperature should provide a clue as to the meaning of this observed difference in virus concentration.

Following the successful adaptation of the mouse line of the SEEREY virus in CFW, CF1, and NIH mice, efforts were made to obtain adaptation of the egg line of the SEEREY virus in CFW and NIH mice. The data included in Table 6 show that adaptation has been observed to occur in CFW mice but not in NIH mice. Employing the same concentration of the SEEREY virus two separate passage series were carried out both in NIH and in

CFW mice. In CFW mice definite pulmonary consolidation was present at the sixth passage in one series and at the eighth passage in the other series. In contrast, in the NIH mice both passage series were negative for virus from the third through the seventh serial passage when 10^{-1} dilutions of the mouse lungs were inoculated in eggs. No further serial passages were carried out in the NIH mice. In another experiment an egg line of the McGuire strain of Type A prime influenza virus which was isolated in New Haven in 1947 and which is antigenically indistinguishable from the seerey strain has been found to adapt in CFW mice at room temperature after eight serial passages. Studies are underway to ascertain the behavior of this strain of virus in NIH mice.

Table 6

Adaptation of the Egg Line of the Seerey Strain of Influenza Virus in CFW Mice

	% Pulmonary consol.				*log EID_{50}*			
*Passage**	*NIH*		*CFW*		*NIH*		*CFW*	
0	<5	<5	5	5				
1	<5	<5	<5	<5	2.7	2.7	5.0	6.3
2	<5	<5	<5	<5	<1.0	>1.0		
3	<5	<5	<5	5	<1.0	<1.0		
4	<5	<5	15	5	<1.0	<1.0	5.2	5.5
5	<5	<5	<5	15	<1.0	<1.0		
6	<5**	<5**	<5	30	<1.0	<1.0		
7			10	70†				
8			35	60†				
9			55	60			5.0	5.8

* Serial passage of the mouse lungs was carried out at four-day intervals.
** Serial passages in the NIH mice were not continued after this passage.
† Some mice died during these passages.

The failure of the egg line of the seerey virus to survive in NIH mice has been observed repeatedly. In a group of experiments with the egg line of the seerey virus, which are still in progress, the virus has persisted for more than three serial passages in NIH mice in only one of twelve tests. In this latter instance the virus is still present after eight serial passages, but signs of pulmonary consolidation have never been observed. The eleven negative tests have remained negative through seven serial passages when they were discontinued. Other findings indicate that the egg line of the seerey virus is more likely to survive on serial passage in NIH mice at room temperature than in mice kept at 5° C. This is paradoxical in view of the results recorded in Table 4 which indicate that four days after inoculation of the seerey virus higher concentrations are attained in the NIH mice maintained at 5° C. than in NIH mice at room temperature. It is

apparent that much future work will be required to resolve some of the problems which have arisen during the course of the present studies.

Finally, brief mention should be made of the finding that the egg infectivity of lungs obtained from Yale University mice that received an intranasal instillation of a small amount of RDE four days after the inoculation of the SEEREY virus was found to be appreciably higher than was anticipated when the mice were sacrificed seven days after receiving the virus. Serial passages of CFW mouse lungs infected with the mouse line of the SEEREY virus were carried out at seven-day intervals. At each passage approximately 0.1 unit of RDE was given intranasally four days after the virus. Extensive pulmonary consolidation was present at the third serial passage and the majority of the mice died within seven days after the second passage. No conclusion concerning the specific effect of RDE may be deduced from these findings. Even without RDE, adaptation of the virus would be expected to occur in CFW mice. Current experiments are concerned with the effect of RDE and inactivated RDE on serial passage of the egg line of the SEEREY virus in NIH mice at room temperature. If RDE exerts any enhancing action on the process of adaptation of the virus, the use of the egg line of the virus in NIH mice should permit its detection.

Summary

Adaptation of the SEEREY Type A prime strain of influenza virus to mice is influenced by the previous passage history of the virus, the genetic background of the mouse, and to some extent by the environmental temperature at which the mice are maintained after inoculation of the virus. The unadapted mouse line of the virus adapts in the inbred CFW mice, the inbred CF1 mice, and the heterogenetic NIH mice. When the environmental temperature of the NIH and CFW mice is lowered to 5° C., adaptation of the virus to these mice occurs more rapidly than at room temperature. Following serial passage of mouse lungs infected with the egg line of the SEEREY virus, the virus becomes lethal, causing extensive pulmonary consolidation in CFW mice. When the egg line is passed serially in NIH mice, the virus in most instances fails to survive for more than a few passages and does not become adapted.

References

1 Burnet, F. M. and Stone, J. D.: The receptor-destroying enzyme of V. cholerae. Austral. J. Exp. Biol., 1947, *25*, 227.

2 Davenport, F. M.: Alternate passage of influenza virus. A comparison of the effect of serial passage and alternate passage of Type B influenza virus in hamsters. J. Immun., Balt., 1951, *67*, 83.

3 Davenport, F. M. and Francis, T., Jr.: A comparison of the growth curves of adapted and unadapted lines of influenza virus. J. Exp. M., 1951, *93*, 129.

4 Friedewald, W. F. and Hook, E. W., Jr.: Influenza virus infection in the hamster. A study of inapparent infection and virus adaptation. J. Exp. M., 1948, *88*, 343.

5 Hirst, G. K.: Studies on the mechanism of adaptation of influenza virus to mice. J. Exp. M., 1947, *86*, 357.

6 Horsfall, F. L., Jr.: Neutralization of epidemic influenza virus. The linear relationship between the quantity of serum and the quantity of virus neutralized. J. Exp. M., 1939, *70*, 209.

7 Jones, M.: Adaptation of influenza B virus to mice by use of certain adjuvants. Proc. Soc. Exp. Biol., N. Y., 1950, *75*, 309.

8 Reed, L. J. and Muench, H.: Simple method of estimating 50 per cent endpoints. Am. J. Hyg., 1938, *27*, 493.

9 Sabin, A. B.: Genetic, hormonal and age factors in natural resistance to certain viruses. Ann. N. York Acad. Sc., 1952, *54*, 936.

10 Salk, J. E.: A simplified procedure for titrating hemagglutinating capacity of influenza virus and the corresponding antibody. J. Immun., Balt., 1944, *49*, 87.

11 Sugg, J. Y.: An influenza virus pneumonia of mice that is non-transferable by serial passage. J. Bact., Balt., 1949, *57*, 399.

12 Sugg, J. Y.: The variation of antigenic pattern and of mouse virulence in an influenza virus culture. J. Bact., Balt., 1949, *58*, 399.

13 Sugg, J. Y.: The relation of the concentration of unadapted and adapted influenza virus in the mouse lung to the death or survival of the infected host. J. Bact., Balt., 1950, *60*, 489.

14 Wang, C-I.: The relation of infectious and hemagglutination titers to the adaptation of influenza virus to mice. J. Exp. M., 1948, *88*, 515.

ROENTGENOLOGICAL ASPECTS OF RENAL FUNCTION*

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During the past twenty-five years excretory urography has become increasingly important as a routine screening technique for disease of the urinary tract. It is used as a case-finding procedure and has been recommended as a test of renal function. A number of iodinated organic compounds have been made available as opaque contrast media which are excreted by the kidney and result in sufficient opacification of the urine on x-ray examination to permit visualization of the renal collecting system.

A new iodinated compound (Urokon)[11] has properties that include low toxicity and high iodine content producing greater opacity to x-rays than any heretofore available. Clinical trials have shown that this compound is well tolerated intravenously and is rapidly excreted by the kidneys. As a sodium salt of 3 acetylamino-2,4,6 tri-iodobenzoic acid in 30 per cent solution it has been found to give excretory urograms of at least equal density to those obtained with other commonly used compounds and results in significantly fewer unpleasant reactions than obtained with other compounds commercially available. As a 70 per cent solution manufactured for angiography, it produced opacification of the renal shadow and abdominal arteriograms[10] after intravenous injection by the Robb-Steinberg[8] technique. Although the incidence of unpleasant side reactions was increased by injections of approximately 50 cc. of the concentrated solution, the percentage of good-to-excellent pyelograms equal in quality to those obtained by retrograde urography was increased from 64 to 79 per cent of the cases.[4]

By the addition of a tracer of radioactive material to the 70 per cent solution for intravenous injection and using a scintillation counter[7] to signal the time of arrival of the contrast material in the femoral artery, we have been able to secure satisfactory arteriograms and nephrograms. In dosages of 25 cc. of the 70 per cent solution approximately 2,500 excretory urographic examinations have been carried out with better visualization of the renal shadows and renal collecting systems than heretofore obtained with lesser concentrations of contrast media.

In order to determine the mechanism of renal excretion at these high dose levels of this contrast medium and to further test its toxicity, Porporis and Mueller[9] have carried out clearance studies in dogs. At dose levels of 0.5-5 gms. per kilo they have been able to show clearance rates similar to those of inulin. Although depression of the para-amino-hippuric acid clearance sug-

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Received for publication March 11, 1953.

gests the possibility that some tubular secretion may occur, the major portion of excreted Urokon is effected by glomerular filtration. Renal clearance studies of Diodrast[2, 9] have indicated that this contrast medium is excreted as a glomerular filtrate as well as by tubular secretion. By tubular absorption of water the contrast medium becomes concentrated and its appearance in the renal pelvis as a dense shadow has been taken as a measure of renal function. Roentgenologists have reported their findings in terms of increased density of the renal shadows, appearance time of contrast media in the collecting system, and evaluation of density of the excreted urine in terms of "Diodrast clearance"[15] or "renal function by excretory urography." The dose of contrast medium for intravenous injection has generally been standardized on the basis of one cc. of 35 per cent Diodrast per 4.4 pounds of body weight. In order to obtain adequate density of contrast medium in the collecting system, a single injection technique is employed so that the level of plasma concentration is on a falling curve during the roentgenological examination. The amount of Diodrast filtered is directly proportional to plasma concentration, but tubular excretion is more variable. Tubular excretion of Diodrast increases proportionally to plasma concentration as long as this is low. When the tubular excretion reaches a maximum of 25-50 mg. per minute, a "depression limit" is reached and very high concentrations of plasma Diodrast may block the tubular secretion completely.[1] In nephritic states the depression limit may be lower than in a normal kidney.[8]

It seems clear from the extensive studies carried out on renal clearance of Diodrast that our evaluation of renal function from single injections of contrast media reflects primarily the ability of the kidney to excrete the contrast media as a glomerular filtrate. Little or no tubular secretion of the contrast media is reflected in the density of the material in the renal pelvis or bladder.

In an effort to improve the quality of the pyelogram it has become standard practice to prepare patients for excretory urography by a period of dehydration for 12 hours prior to the examination. In general this procedure has resulted in better shadows of the collecting system when relatively low plasma concentrations have been reached after intravenous injection of 30-35 per cent solutions of contrast media. However, two important factors affecting the quality of the desired anatomical detail of the renal pelvis are density of the contrast material and distension of the collecting system by the opacified urine. A frequent observation on excretory urograms is good visualization of one renal pelvis of large capacity and fragmentary or incomplete visualization of its small capacity fellow on the opposite side. Increased peristalsis, irritability, or poor function are often suggested as the explanation for poor visualization. We have occasionally noted non-visualization of one renal pelvis on several of the serial films as the result of over-prompt emptying of a small capacity pelvis. A repeat examination with moderate hydration has resulted in greater distension of both

renal pelves and consequently more satisfactory pyelograms as the result of maintaining adequate urine flow during the period of examination. With high plasma levels of iodinated compounds, dehydration to obtain adequate contrast density in the renal pelvis becomes less important than maintenance of urine flow to obtain adequate distension of the collecting system. The use of ureteral blocks to obtain this distension has been criticized as disturbing normal renal excretion. Avoidance of the dehydration period or controlled hydration by giving water to the dehydrated patient one hour prior to intravenous injection of concentrated contrast medium has been shown to improve the shadows of small renal pelves by insuring adequate urinary output during the period of roentgenological examination.

Since the level of plasma concentration largely determines the concentration of contrast medium in the glomerular filtrate of normal kidneys after single injection techniques, it seems advisable to avoid rigid standardization of dose for intravenous injections. We have found that a dose based upon the formula of 15 cc. of 70 per cent solution of Urokon Sodium per square meter surface area results in more uniformly satisfactory routine urograms than a formula based on body weight.

The appearance time of concentrated contrast media in the collecting system of normal kidneys is approximately three minutes. A five-minute roentgenogram usually demonstrates the best definition of anatomical detail of the renal collecting system. Throughout the routine serial films there is seen an increased density of the renal shadow as compared with the density seen on a control scout film before injection of the contrast material. The density of the nephrogram becomes greater with partial ureteral obstruction but is much less dense than the immediate nephrogram seen after intravenous injections of large doses for arterio-nephrography. Properly timed exposures after intravenous injection of 50 cc. of Urokon will give good renal arteriograms and a brilliant nephrogram that we interpret as demonstrating the capillary network of functioning renal parenchyma. The narrow band of renal cortex is denser than the medulla. Occasionally the renal veins can be seen. Opacification of the liver and spleen is also usually obtained. Simultaneous with the appearance of contrast material in the collecting system there is fading of the dense nephrogram. Opacification of liver, spleen, and kidney suggests that most of the density is due to contrast media in the blood capillaries. The greater intensity of the renal shadow and its persistence on serial films probably indicates a large component of transport of glomerular filtrate in the collecting tubules. It has not yet been possible to determine the precise contribution due to contrast material in blood and in the tubules. However, the value of the immediate nephrogram as an indication of functioning renal parenchyma seems clear from the clinical studies that have been made. Avascular renal cysts may be differentiated from vascularized cortical neoplasms. Experimental reduction of blood flow by clamping a renal artery will reduce the density of the nephrogram

on the affected side. Experimental hydronephrosis from tying off a ureter will also result in marked decrease in the density of the immediate nephrogram on the affected side as compared with the normal kidney.

The roentgenographic visualization of the kidney and its effectively functioning parenchyma, and the improved quality of pyelograms following the intravenous injection of concentrated contrast media, promises to advance our evaluation of renal function and recognition of renal disease. It is equally important, however, to recognize the limitations of the excretory urographic method in terms of nephron physiology. With high plasma levels of contrast media presently used we can only attempt to judge in a crude fashion the mechanism of glomerular filtration. The function of tubular secretion plays little or no significant rôle in the excretory urogram. Evaluation of water absorption by the tubules is unreliable at the high plasma concentrations of contrast media used to improve the quality of the pyelogram. In spite of these limitations, efforts to determine plasma concentrations, diuresis, and concentrations of urinary excretion of contrast media during excretory urography should prove practical and valuable in the roentgenological evaluation of renal function.

References

1 Eggleton, G. and Habib, Y. A.: Excretion of para-aminohippurate by the kidney of the cat. J. Physiol., Lond., 1950, *110*, 458.

2 Findley, T., Edwards, J. C., Clinton, E., and White, H. L.: Intravenous urography: test of renal function. J. Urol., Balt., 1942, *48*, 119.

3 Josephson, Bertil: The mechanism of the excretion of renal contrast substances. Acta radiol., Stockh., 1952, *38*, Fasc. 4, 299.

4 Nesbit, R. M. and Nesbitt, T. E.: Experiences with high concentration Urokon for pyelography. Univ. Mich. M. Bull., 1952, *18*, 225.

5 Pendergrass, E. P.: Excretory urography as a test of urinary tract function. The Carman Lecture. Radiology, 1943, *40*, 223.

6 Porporis, A. A. and Mueller, C. B.: Urokon and renal function in the dog. (To be published.)

7 Porporis, A. A., Zink, Oscar C., Wilson, H. M., Barry, C. N., Royce, R., and Rose, D. K.: Routine clinical experiences using Urokon sodium 70% in intravenous urography. (In press)

8 Robb, G. B. and Steinberg, I.: Visualization of chambers of the heart, pulmonary circulation and great blood vessels in man. Am. J. Roentg., 1939, *41*, 1.

9 Smith, H. W.: *The kidney. Structures and function in health and disease.* New York, Oxford University Press, 1951.

10 Wall, Bithel and Rose, D. K.: The clinical intravenous nephrogram: Preliminary report. J. Urol., Balt., 1951, *66*, 305.

11 Wallingford, V. H., Decker, Harriet G., and Kruty, Margaret: X-ray contrast media. I. Iodinated acylaminobenzoic acids. J. Am. Chem. Soc., 1952, *74*, 4365.

CLINICAL STUDIES IN INFERTILITY

A Statistical Survey*

EMIL D. KARLOVSKY AND HERBERT THOMS

The Infertility Clinic was established in the Department of Obstetrics and Gynecology in 1946, the first couple being enrolled on April 12th. From that time to June 30, 1952, 681 couples were enrolled. The records show 153 pregnancies in this group and 510 couples to have been discharged. The incidence of pregnancy in the entire group is 22.5 per cent, a figure which has changed to 27.5 per cent for the last three years. Many of the couples, listed as discharged, voluntarily discontinued their visits, and follow-up in a considerable number of these has not been satisfactory. Additional pregnancies in the group are probable although unknown to us.

We have follow-up records of 230 couples in which 54 pregnancies occurred. Of the 176 couples of this group in which pregnancy has not occurred we are aware of a cause or of causes in 38 couples which we believe contributed to infertility. Analysis of data in this unsuccessful group shows:

1. Average duration of exposure to pregnancy 6.8 years, spanning 1-20 years.
2. Average age of female partner 30 years, spanning 21-41 years; 7 women were over 35 years.
3. Tests for determining tubal patency
   insufflation 21 times in 16 women—patency established in one instance;
   salpinograms in 19 women—patency established in one instance.
4. *Tubal occlusion* was present in 18 women. In one woman laparatomy was performed at which tuberculous salpingitis was discovered. In one woman multiple myomata were present and in five women bilateral hydrosalpinx was diagnosed.
5. *Basal metabolism* rates were determined in 25 women. The lowest rate was —30 and in six instances it was below —15.
6. *Endometrial biopsy.* Specimens for biopsy were taken from four women. All were reported normal.
7. *Other complications.* On investigation it was found that two women of this group had had both fallopian tubes removed. They were unaware of the extent of the surgery done and had not been told of the impossibility of the occurrence of pregnancy. Two women had epilepsy and were advised against the continuance of infertility studies. One woman and her husband were diabetic. One woman had a known psychosis and was advised against childbearing. One woman had amenorrhea of two years' duration which was uncorrected after intensive therapy. The previous history of these women revealed three had had appendectomies, four had known chronic inflammatory pelvic disease, one had had a thyroidectomy.

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Received for publication February 19, 1953.

Male Factors in Infertility

The source of infertility was believed to be related to the male partner in 13 couples. Eight men showed complete aspermia. Six men had a marked degree of oligospermia. One man had severe chronic prostatitis. One man had urethral stricture.

Of the remaining 138 of the 230 couples, none completed the basic program of investigation of this clinic. On analysis it was found that 7 per cent of the couples had moved away from the area, 3 per cent discontinued visits because of financial factors, 12 per cent transferred to a private physician, 7 per cent made arrangements for adoption and discontinued clinic visits, and 61 per cent discontinued visits for reasons undetermined by us. A goodly number of this latter group made but a single visit to the clinic which is not an uncommon experience in clinics of this kind.

A survey of certain data concerning 40 couples in which pregnancy was established during or following their period of clinic attendance shows that the average duration of childlessness was 3.3 years. In 10 couples this period was 5-8 years. The average age of the women was 27 years, youngest 19, oldest 38. Pregnancy occurred from 1 to 28 months after the initial visit. In 11 instances pregnancy did not occur for one year following. The number of visits varied from 1-22, average 6.4 visits per couple.

Factors relating to infertility in these 40 successful couples are summarized. In five couples factors were divided between the partners.

Male. Twelve men were found to have some degree of oligospermia. Eight were referred to a urologist and all showed response to therapy.

Female. Twenty females were found to have conditions which could have been contributory to their infertility.

	Tubal factor	2
	Pelvic Inflammatory Disease	6
	Cervicitis	7
	Erosion	9
	Retroversion	8
	Cervical polyp	2
	Anovulatory menstrual cycles	4
	Trichimonas infection	3
	Monilia infection	1
	Overweight	8
	Underweight	2
	Myomata uteri	1
	Anemia	3
	Anteflexion	1
Previous Surgery:	Appendectomy	11
	Ovarian cyst resection	4
	Oophorectomy	2
	Ectopic pregnancy	2
	Uterine suspension	2
	Salpingectomy	2

Comment

In general our experience parallels that of reports from other clinics; namely, infertility factors are present in the male partner in 30-40 per cent of childless couples seeking clinic advice, and divided between the partners in 60-70 per cent. Somewhat over one-half of couples coming to our clinic had consulted a physician previously about their situation. In 10-15 per cent of our couples endocrine imbalance seemed to be related to the infertility. We are convinced also that psychogenic factors play an important rôle and should be given more consideration. Problems in these areas can certainly be aided in their solution by the recent establishment of the endocrine laboratory and an anticipated greater coöperation with the Department of Psychiatry.

ELECTRICAL CORRELATES OF OVULATION IN THE RHESUS MONKEY*

VINCENT L. GOTT† AND H. S. BURR

It has been generally agreed by most investigators that ovulation in the higher primates occurs in the middle of the menstrual cycle. In humans, the ovulation period is believed to fall on or about 14 days, plus or minus twenty-four hours, before the onset of the succeeding menses. The test for ovulation has been records of mating, with subsequent pregnancy, or the recovery of young ova in elective laparotomy.

Many studies have been made on ovulation in the monkey, notable among which are the papers of Hartman,[8] VanWagenen,[16] and Allen.[1] Hartman reported ovulation in rhesus between the 11th and 14th day of the menstrual cycle. VanWagenen's more recent study reported that the 11th and 12th days constituted the critical period. Most of the studies commonly quoted indicate the approximate time of ovulation. Pragmatically, determining with certainty the moment of follicular rupture may not be very important. However, if it were known accurately, the age of the corpus luteum could be ascertained within hours, if not minutes.

Burr and Musselman[4, 5] noted a series of interesting potential changes between the index fingers in women, followed through successive menstrual cycles. Sharp rises in potential difference occurred during the period when ovulation in women is believed to be most probable. These facts were subsequently confirmed by Barton.[2] Paralleling these studies, Burr, Hill, and Allen[3] undertook to determine the validity of potential changes during ovulation in the rabbit. In this mammal, ovulation is believed to occur from 9 to 12 hours after mating. An electrical study of this period revealed a sharp rise in potential difference between vagina and symphysis pubis. This was confirmed at laparotomy shortly after cessation of the voltage changes. In one instance, with the ovary inclosed in a suitable chamber where it could be observed with the microscope, a developing follicle was watched while electrometric determinations were being made. As the follicle increased in size, the potential rose and reached a peak at the instant of rupture. This was followed by a decline in the voltage gradient. Subsequently Reboul, Friedgood, and Davis[12] confirmed these findings and contributed the fact that various stab wounds of abdominal viscera produced no such changes in electrical properties.

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† Based on a thesis presented in partial fulfillment of the requirements for the degree of Doctor of Medicine, Yale University School of Medicine, 1953.

Received for publication April 6, 1953.

Burr, Musselman, Barton, and Kelley[6] examined these relationships in a patient requiring an elective laparotomy. A continuous electrical record was made from this patient, beginning at a time when previous voltage gradients had indicated the probability of ovulation, and continued for 57 hours. Toward the end of this period, there was a gradual change of the voltage gradient from 25 millivolts, with vagina positive, to approximately 25 millivolts with vagina negative. Following this, there was an abrupt rise in potential with a subsequent return to a vagina positivity, but with a noticeable increase in moment to moment variability. This last phenomenon had been observed in rats by Boling (unpublished). At laparotomy, a recently ruptured follicle was observed. A portion of the ovary was removed and examined microscopically, confirming the fact of ovulation.

Meanwhile, Rock had been studying this problem and confirmed the findings, but generously withheld publication until Burr and his associates had had an opportunity to publish their results. Rock, Reboul, and Wiggers[10] and Rock, Reboul, and Snodgrass[14] published their results. For reasons which are not clear, Rock[13] in 1941 reversed his position, holding that other factors must be responsible for voltage changes.

These facts made it abundantly clear at that time that further investigation could be best forwarded by a study of the electrical correlates of ovulation in the monkey. Many abortive attempts were made with wild monkeys recently brought to the laboratory. The attempts had to be abandoned because of the wildness of the animals. Since repeated observations were required, anesthesia could not be used and confinement in a restraining box yielded no adequate results because of the excitement of the animals.

Recently, through the courtesy of Dr. VanWagenen of the Department of Obstetrics and Gynecology, Yale University School of Medicine, one of us (V.L.G.) was given the opportunity of seeking a solution to this problem, using animals bred in captivity for several generations. The animals were so tame that they lay quietly on the table and showed no undue excitement during the determinations.

In organizing the experimental work, two interesting aspects have been developed for investigation.

1. Although there has been extensive work done in other animals and quite a bit of work in the human on the electrical detection of ovulation, none has been done in the rhesus, the best experimental animal of all for this work. The work here reported on rhesus throws additional light on ovulation and its electrical detection.

2. By taking daily readings in six monkeys over several months, it was possible to get an electrical record of ovulation and, in two animals, of pregnancy from the day of conception until delivery. A check of the electrical record throughout pregnancy was made to see if there is a significant

pattern a few days after conception which might allow detection of pregnancy at this early date.

The equipment used to record the voltages consisted of the Burr-Lane-Nims vacuum tube voltmeter and two silver-silver chloride electrodes. A complete description of this equipment may be found in a paper published in 1936 by Burr and Musselman.[8]

The problems to be encountered in recording the potential difference between the vaginal readings require that they should be taken every day throughout the menstrual cycle for several cycles. If the pregnancy is to be detected, daily readings must be recorded from the day of conception in order to pick up any consistent alteration in the electrical pattern. It was necessary, therefore, to take daily or almost daily readings on the monkeys for upwards of ten months. The rhesus is a fairly excitable animal and it was apparent that a method would have to be devised for keeping the monkeys perfectly quiet while the vaginal and abdominal electrodes were properly placed and the potential difference recorded. The tameness of the animals used made it possible to record the potential differences between the vagina and three points on the surface of the ventral abdominal wall—one in the midline over the fundus, one over the right ovary, and one over the left ovary. The positions of these three organs were determined by previous rectal-abdominal bimanual palpation, and marks with gentian violet were made over the organs on the skin but 1 cm. cephalad in each case, so the electrode would not have to be placed on the gentian violet. Before placing the abdominal electrode on the skin, the skin was moistened with a cotton swab soaked in normal saline. With the vaginal electrode in place, the abdominal electrode in the first of its three positions, and the animal lying quietly, the reading on the voltmeter was noted and recorded. The electrodes were usually left in place for a minute or until the needle came to rest. If the needle is deflected to the left, the vagina is negative in respect to the anterior abdominal wall, and if the needle is deflected to the right, the vagina is positive in relation to the point on the anterior abdominal wall where the abdominal electrode rests. The number of millivolts with the needle deflected to the left or right of zero was recorded and termed as negative or positive, whichever the case might be. After recording the voltage with the abdominal electrode over the left ovary, the area over the fundus was moistened, the electrode placed on this point, and the reading noted after the needle deflection stopped. The procedure was again repeated with the abdominal electrode over the right ovary.

Readings were taken in this manner throughout the menstrual cycle and in three cases throughout pregnancy. After the first month, there was little variation in the electrical pattern, so it was necessary to take readings only about once a week during the latter stages of pregnancies. While the electrical pattern of the menstrual cycle was being recorded, the cycle itself

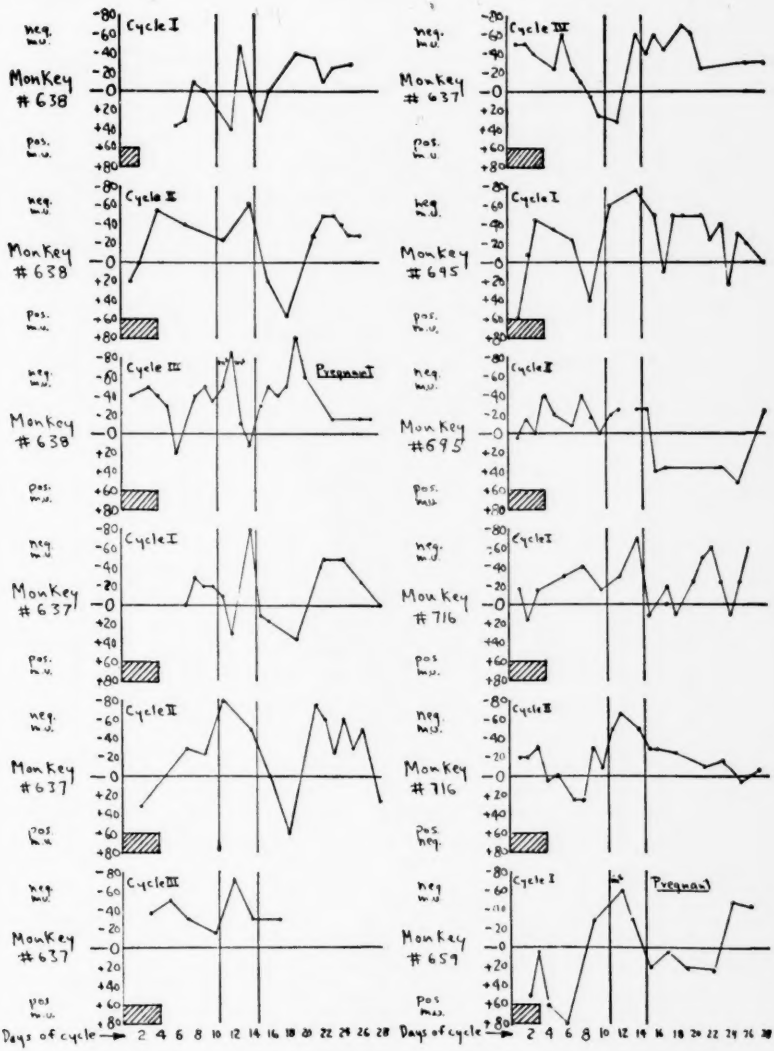


FIG. 1. A graph of changing potential differences between the mid-ventral abdomen and the vagina in twelve menstrual cycles of rhesus. Cross-hatched blocks indicate periods of bleeding.

was being accurately timed by following the period of menstrual bleeding with the eye-dropper technique.

A summary of the electrical records of twelve cycles is shown in Figure 1. The midline-vagina potentials only are plotted since the ovary-vagina gradients are, for all practical purposes, identical with them.

It will be noted that the electrical pattern in twelve ovulation periods show peaks of high negativity in ten of the twelve cycles. Only in cycle two of ♯695 and cycle four of ♯637 are there no significant peaks. Further, it should be noted that there are peaks which occur on other days in the cycle. However, those occurring between the 11th and 14th days are significantly different. In the first place, all ten of the mid-cycle peaks are higher than are other voltages during that particular cycle, except in one instance when, in the third cycle of ♯638, a high peak of negativity was registered 19 days after the beginning of the cycle and seven days after the ovulatory peak that occurred on the 12th day. This post-ovulatory peak attained a voltage of —100 m.v., whereas the peak occurring on the 12th day only reached —80 m.v. This animal, however, proved to be pregnant at the time this —100 m.v. peak occurred on the 19th day. The high voltage peak in this instance, therefore, could not be truly considered an evidence of ovulation but a voltage peak occurring during pregnancy, the animal being seven days' pregnant at the time of this high peak. An examination of individual records reveals that there are several peaks not occurring during the 11th to 14th days that are almost as high as the mid-cycle peaks. To further distinguish the mid-cycle peak from others, however, it will be noted that it is not only the highest during that particular menstrual cycle, but different in pattern and design from the rest. It is the only peak in each of the ten cycles that begins either at a voltage that is positive (below the zero line) or from a low negative voltage near the zero line and rises to an extremely high negative voltage, then abruptly dropping in nine cases out of ten to a voltage below the zero line or to a positive voltage. In one instance (♯637, cycle three) readings were missed on the 15th and 16th days when one might assume that the peak continued its downward course to below the zero line.

It is the rapid and abrupt drop from the highest peak of negativity in the cycle to zero line that is the consistent and significant factor in distinguishing the ovulatory peak from the rest.

It is apparent that in ten cycles out of twelve, a peak that is consistent in pattern and time of occurrence can be readily distinguished from the other peaks in the cycle.

In cycle one of monkey ♯716, there is a high peak of negative voltage on about the 22d day that is quite similar to the mid-cycle peak. The mid-cycle peak, however, is slightly higher. Since the ten significant and consistent peaks all occur between the 11th and 14th days, when 85 per cent of

monkeys ovulate, it may be concluded that these peaks are the electrical correlate of ovulation.

What the electrical activity may represent in more specific terms is largely unknown, but it is assumed that this is a recording primarily of ovarian function. More specifically, it is possible that the rise in negative voltage is the increased activity of the follicle before rupture, and the abrupt drop in voltage to below the zero line might represent the period after the ovum is extruded and the follicles decrease in activity.

An explanation of the two cycles in twelve (♯695 cycle two, and ♯637 cycle four) that had no ovulatory peak may lie in the fact that the rhesus may have anovulatory cycles. This is a fairly common occurrence. In ♯637 cycle four, it will be noted that the voltage increases in negativity but there is never an abrupt drop to below the zero line. Voltages were taken each day from the 12th to the 17th and it is felt that if ovulation had occurred in this cycle, there would have been a significant drop to the zero line.

In cycle three of ♯695 there is no significant ovulatory peak. Here again this might represent an anovulatory cycle, but the reading on the 13th day was missed. This could have been the high voltage that would have turned the voltage plateau into a peak.

Readings were taken over the left ovary, fundus, and right ovary with the common electrode on the cervix with the thought in mind that there might be different patterns from these three skin areas. Possibly the pattern of voltage picked up from the electrode over the ovulating ovary might be significantly different from the pattern of the other two skin areas. However, we are unable to find any significant difference in the three records that might indicate which ovary is ovulating. The potential differences recorded from the three electrode positions were all approximately of the same magnitude, and the three electrical patterns were similar.

The patterns throughout the rest of the cycle (other than the ovulatory period) did not seem to run a consistent course. The pattern during bleeding was closely surveyed and there is apparently nothing significant in the electrical picture at that time. The only consistent and significant pattern recorded is the ovulatory peak that occurs between the 11th and 14th days.

Pregnancy in the female rhesus is similar to that of the woman in that it is a pregnancy of long gestation, being 168 days in length (six months). The development of the rhesus embryo is very similar to that of the woman. It is in the latter parts of pregnancy that the rhesus fetus develops more rapidly than the woman, accounting for the shorter gestation period.[7, 9, 10]

Each animal was mated during the midportion of each cycle in the hope of a resulting pregnancy. This mating was done during twelve menstrual cycles in five animals and only two animals became pregnant. In animal ♯638 abdominal-vaginal voltages through two cycles were recorded, both having ovulatory peaks. On the third recorded cycle, the monkey again displayed a good ovulatory peak on the 12th day, and was mated on the

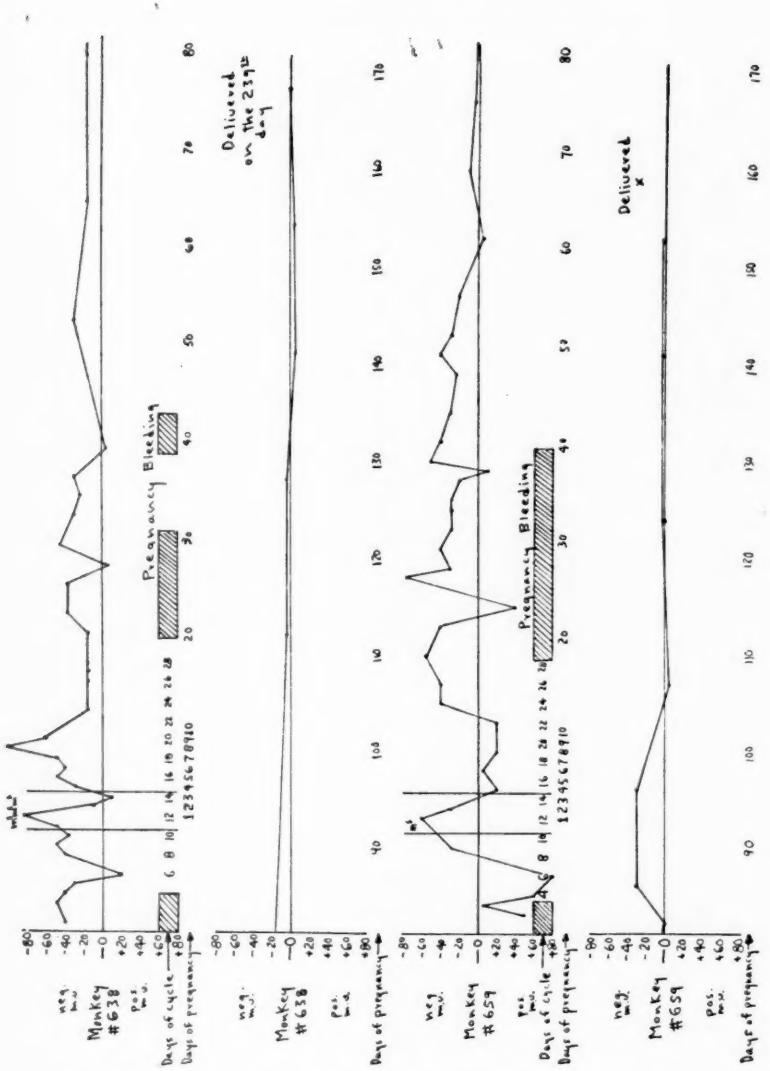


FIG. 2. A graph similar to Figure 1 during two pregnancies.

11th and 12th days. The voltages during this cycle were recorded and on the 31st day of this cycle she commenced to bleed and continued to do so for ten days. This bleeding is the pregnancy bleeding which is common to monkeys and indicates pregnancy. Readings were continued every three or four days throughout her pregnancy, for 168 days. She was delivered 239 days after conception.

With animal ♯659, abdominal-vaginal voltages were recorded shortly before her menstrual bleeding began and continued throughout the following cycle. A good ovulatory peak appeared on the 12th day of the cycle. The animal was mated from noon on the 11th day until noon on the 12th day, and sperm was found in the vagina after this mating. The recording was continued through this cycle and on the 29th day the animal began to bleed and bled for 21 days, which indicated she was pregnant. Readings were taken bi-weekly and weekly throughout her pregnancy, until she was delivered 159 days after conception.

In the records of these two animals (Fig. 2) it should be noted that in ♯638 there is an extremely high peak of negativity on the eighth day following conception and this is the only case in which a peak of negativity is higher than the ovulatory peak of the corresponding cycle. The voltage pattern drops after this high peak and, except for the positive readings on the 27th and 39th days, remains negative for three and two-thirds months. The pattern of this negative voltage of pregnancy is more level than the mountainous pattern of the menstrual cycle. In fact, it will be noted that the record is almost a straight line from the 60th day, or second month of pregnancy, throughout the readings, until they were stopped on the 168th day. The voltage remains slightly negative until about the 110th day of pregnancy, when it hovers near the zero line. This leveling off of the pregnancy voltage is certainly different from the highly alternating voltage of the menstrual cycle.

In ♯659, the voltage becomes positive following ovulation and conception, and remains positive for about ten days after conception when it then becomes negative and remains negative, except for two instances on the 23d and 37th days of pregnancy, until the 75th (two and a half months) day, when it reaches zero and remains near this line until delivery.

One of the questions in this study was to examine the voltage record shortly after conception to see if there is a significant and consistent change in the pattern that might indicate pregnancy. It is apparent that in ♯638 an extremely high peak of negativity appeared on the eighth day following conception. This is the only instance in all the ten menstrual cycles in which there is a peak of negativity higher than that occurring during the ovulatory period. However, the pattern of ♯659, immediately following conception, is completely different from that of ♯638 and so, in two animals at least, there is no consistency in the early patterns after conception. Two pregnant animals are not enough to solve this problem of the possibility of detecting

pregnancy by this technique. However, in twelve attempted impregnations over twelve monthly cycles, only two animals were successfully impregnated. Although there is no consistent pattern in these two animals shortly after conception that might indicate pregnancy, it is hoped that more recordings eventually may be taken so that this problem can be elucidated more thoroughly. Possibly the reason for such inconsistent patterns shortly after conception may be attributed to the fact that there are several very active functions taking place at the same time that may confuse the picture. There is not only the great activity of the growing embryo, with its electrical correlate but, simultaneously, the placenta is actively developing in the uterus and the corpus luteum is growing in the ovary. Possibly these activities have electrical correlates. With pregnancy in only two animals, it was decided to read a third animal who was approximately 31 days' pregnant and who had not been read previously (#639). This, of course, is too late for the recording of an early pregnancy but the pattern of the last five months of pregnancy might be interesting. The voltage remained negative (except for the 37th day) until the 110th day when it dropped to near the zero line where it hovered throughout the remaining portion of pregnancy until delivery. We thus see that in all three pregnancies the voltage remains negative from about the first month until approximately the third month when the voltages drop to the zero line where they hover throughout the remaining period of pregnancy.

It is clear that there is no consistent pattern a few days after fertilization in two animals that might permit a diagnosis of pregnancy. However, it is apparent that the pattern, after the 30th day of pregnancy, is definitely distinct and different from that of the menstrual cycle in that it levels out somewhat and the cervix remains negative until about the middle of the third month when it approaches a zero potential and remains close to the zero line throughout the remaining three months of pregnancy. These records closely parallel those described in the human by Burr and Musselman.[5]

Summary

Voltages were taken daily in five rhesus females for a total of twelve menstrual cycles. It was found that in ten out of twelve cycles there was a significant and consistent peak of negative voltage that was higher than any peak in the cycle and because of its distinctive pattern and size may be distinguished from any of the other peaks in the cycle. This distinctive peak occurred in every cycle between the 11th and the 14th day and this is the period during which most of the ovulations are known to occur. It is believed that this distinctive and reproducible peak is the electrical concomitant of ovulation.

Possibly the two cycles without a significant peak between the 11th and 14th days are anovulatory cycles, which are fairly common in the monkey.

Bi-weekly or weekly records of the abdominal-vaginal potential difference

were made in two monkeys from the day of conception until delivery six months later. In one other rhesus, records were taken from the second month of pregnancy until delivery. The record tends to level out somewhat, as pregnancy proceeds, so that by the third month the graph is almost a horizontal line. The cervix remains negative after the second week of pregnancy until the beginning of the third month, except for rare instances, when it becomes positive. By the third month, the voltage has dropped to near the zero line, where it hovers until delivery. Despite these characteristics, which differentiate pregnancy from the menstrual cycle, there were no significant and consistent changes during the first month of pregnancy that might permit the detection of pregnancy a few days after conception.

It is hoped that further data can be added to the foregoing so that this problem may be more thoroughly and completely elucidated.

References

1 Allen, E.: The time of ovulation in the menstrual cycle of the monkey (Macaque) rhesus. Proc. Soc. Exp. Biol., N. Y., 1926, *23*, 381.

2 Barton, D. S.: Electric correlates of the menstrual cycle in woman. Yale J. Biol., 1940, *12*, 335.

3 Burr, H. S., Hill, R. T., and Allen, E.: Detection of ovulation in the intact rabbit. Proc. Soc. Exp. Biol., N. Y., 1935, *33*, 109.

4 Burr, H. S. and Musselman, L. K.: Bioelectric correlates of the menstrual cycle in women. Am. J. Obst., 1938, *35*, 743.

5 Burr, H. S. and Musselman, L. K.: Bioelectric phenomena associated with menstruation. Yale J. Biol., 1936, *9*, 155.

6 Burr, H. S., Musselman, L. K., Barton, D. S., and Kelley, N. B.: A bioelectric record of human ovulation. Science, 1937, *86*, 312.

7 Corner, G. W.: Development of the corpus luteum in the rhesus monkey. Contr. Embryol. Carnegie Inst., 1945, *31*, 117.

8 Hartman, C. G.: Studies in the reproduction of the monkey, Macaque Rhesus, with special reference to menstruation and pregnancy. Contr. Embryol. Carnegie Inst., 1932, *23*, 1.

9 Hartman, C. G. and Corner, G. W.: Maturation division of Macaque ovum. Contr. Embryol. Carnegie Inst., 1941, *29*, 1.

10 Heuser, C. H. and Streeter, G. L.: Development of the Macaque embryo. Contr. Embryol. Carnegie Inst., 1941, *29*, 15.

11 Langman, Louis and Burr, H. S.: Electrometric timing of human ovulation. Am. J. Obst., 1942, *44*, 1.

12 Reboul, J., Friedgood, H. B., and Davis, H.: Electrical detection of ovulation. Am. J. Physiol., 1937, *119*, 387.

13 Rock, J.: Ovulation. N. England J. M., 1941, *225*, 910.

14 Rock, J., Reboul, J., and Snodgrass, J. M.: Electrical changes associated with human ovulation. Am. J. Obs., 1938, *36*, 733.

15 Rock, J., Reboul, J., and Wiggers, H. C.: The detection and measurement of the clinical concomitant of human ovulation. N. England J. M., 1937, *217*, 654.

16 VanWagenen, G.: Optimal mating time for pregnancy in the monkey. Endocrinology, 1945, *37*, 301.

EVENTS

MICROBIOLOGY SEMINAR

January 30, 1953

BACTERIAL CYTOCHROMES. By Lucile Smith, Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia, Pennsylvania.

Although Warburg included acetic acid bacteria in the study of his *Atmungsferment* and Keilin established that B. subtilis is characterized by a four-banded spectrum typical of heart muscle or yeast, while B. communis has only two bands without cytochrome oxidase activity, additional information concerning the cytochrome systems of bacteria has been scant.

For sensitive measurement of the absorption spectra of various bacteria in both visible and invisible light, an apparatus has been devised which utilizes the monochrometer of Beckman and by delicate methods of amplification permits the recording of an average optical density change of 0.02. When the oxygen tension of a suspension of respiring cells reaches 0, there is a sharp increase in the optical density, and this difference, plotted against the wave length, provides a characteristic spectrum. In a substrate that gives the maximum respiration rate, oxidase activity is shown to greatest advantage and the cytochrome absorption bands of a wide range of bacteria can be compared.

Few of the organisms studied resemble yeast and of those that do, some may have the yeast spectrum in the visible range but be missing cytochrome a_3 in the soret. It is noteworthy that an intact porphyrin ring is necessary for the soret band. Other bacteria have cytochrome a, or a_2, or a mixture of a_1 and a_2. Cytochrome a_1, a cytochrome oxidase, is frequently observed in bacteria having a high respiratory rate. From all available data it may be concluded that the pigment content of the bacteria, that is, respiration in terms of the cytochromes, approximates or is even greater than that in yeast.

M.-L. J.

CARDIOVASCULAR STUDY UNIT

February 5, 1953

THE USE OF THE FIVE-MINUTE STARLING CURVE AS A MEANS OF QUANTITATING CARDIAC PERFORMANCE. By S. J. Sarnoff, Department of Physiology, Harvard University School of Public Health, Boston, Massachusetts.

These studies were performed on anesthetized, open-chest dogs. Pressures were recorded from cannulated auricles, ventricles, the aorta, and a branch of the pulmonary artery. A continuously registering electroturbidometer recorded the cardiac output. Cardiac performance was calculated as stroke work (SW)

$$SW = \frac{\text{Aortic Pressure} - \text{Left Auricular Pressure}}{100} \times \text{Stroke Volume} \times 1.06,$$

neglecting the velocity factor. Starling's Law applies more accurately to this estimate than to either flow or pressure measurements alone. When coronary flow is included in the calculation, the resulting curve (of SW plotted against rising left auricular pressures) will be somewhat higher in value but parallel to the curve obtained when coronary flow is

neglected. The rate of the increase in auricular pressure does not influence the ultimate rise of cardiac performance.

Administration of suprarenin results in a three-fold increase in SW, with a concomitant drop in left auricular pressure. Continuous recording of performance showed that the drug produced a new relationship, yielding a higher curve, i.e., more work at a lower filling pressure.

Cardiac tamponade also results in phenomena seemingly contrary to Starling's Law, SW decreasing in spite of higher absolute filling pressures. However, the effective pressure is the difference between intra- and extracardiac pressures and in cardiac tamponade it appears that the rise in extracardiac pressure is far in excess of the rise in filling pressure. Hence the dilating pressure actually falls, and thus SW is depressed.

Gradual aortic occlusion shows a possible rôle of the normal pericardium. Under these circumstances elevation of the auricular pressure produces a smaller rise in SW than would be expected. Opening the pericardium eliminates this effect, while tightening it enhances it. Thus, here we are dealing with a pseudo-tamponading effect of normal pericardium on the dilating heart. Since this effect involves the right ventricle also, without a rise in pulmonary artery pressure, the pericardium seems to act to equalize the SW of the ventricles when a differential strain is imposed on the left ventricle.

Decrease of left coronary artery flow produces a drop in left ventricular SW with a concomitant rise in left auricular pressure. This is the result of the heart having shifted to a lower curve of performance for the same auricular pressures, presumably due to anoxia. Restoration of coronary flow reëstablishes the previous, higher curve.

The main conclusions drawn from these experiments were: (i) Starling's Law of the Heart can be experimentally verified; (ii) the filling pressure in the right auricle and the left ventricle SW may not be related, i.e., each ventricle may function on a different level of performance; and (iii) there exist several "Starling's Curves" for each heart chamber, and the heart will function along one or another of these depending on the momentary determinants.

N. A. H.

PHARMACOLOGY SEMINAR

February 5, 1953

SOME INDICATIONS OF CEREBRAL HUMORAL MECHANISMS. By A. S. Marrazzi, Clinical Research Division, Chemical Corps Medical Laboratories, Army Chemical Center, Maryland.

Synaptic areas in the sympathetic and parasympathetic ganglia, and in the optic and auditory transcollosol and respiratory tracts, respond to acetylcholine and epinephrine in a similar manner.

Acetylcholine in doses of 2.5γ stimulated the electrical activity of synapses in the two ganglia but doses of 5γ inhibited it. As in other synapses, atropine acted to block activity, and anticholinesterases increased it. Adrenaline exerted its usual inhibitory action.

The optic tract was stimulated in two ways: by flashing a light into the eye and by an electrical current through electrodes implanted in the tract. The auditory apparatus was stimulated by a ticking watch and the transcollosal tract through implanted electrodes. Drugs were injected into the ipsilateral carotid artery. It was found that acetylcholine in low dosage

increased electrical activity, while larger doses caused inhibition. Anticholinesterases likewise increased activity, while atropine produced an inhibition as did epinephrine, ephedrine, benzedrine, and mesocholine. It was found that the activity of the respective transcollosal tracts was the same qualitatively as that in the tracts from the eye or the ear, despite the fact that the transcollosal pathway has one synapse, while that from either sense organ has five or six.

In the respiratory tract, acetylcholine again stimulated in low doses and inhibited in high. D-tubocurarine was here found to have a central inhibitory effect which was seen at dosage levels that caused paralysis of the diaphragm. The inhibitory effect was reversed by acetylcholine.

P. D. BONNYCASTLE

MICROBIOLOGY SEMINAR

February 6, 1953

REACTIONS OF TRIOSEPHOSPHATE DEHYDROGENASE. By Jane Hasting, Department of Pharmacology, New York University, College of Medicine, New York.

The study of triosephosphate dehydrogenase in a series of reactions has indicated several transfer activities that can be attributed to the enzyme as prepared from muscle. Acetaldehyde, in the presence of DPN, will yield the acetyl enzyme which can be phosphated or arsenated. By adding labelled ($P^{32}O_4^{\equiv}$), ATP, Bücher's enzyme, and 3-phosphoglyceric acid, ATP^{32} is produced by the reversible reactions:

3-phosphoglyceric acid + ATP $\rightleftharpoons$ 1,3-phosphyglyceric acid

1,3-phosphyglyceric acid + triosephosphate dehydrogenase $\rightleftharpoons$ 3-phosphyglyceryl-enzyme + $PO_4^{\equiv}$

A second rôle of the enzyme is shown by its ability to carry out transfer reactions. For example, the acetyl group of acetyl-phosphate can be transferred to glutathione to form acetyl-glutathione, or to CoA to form acetyl-CoA. This latter product will acetylate sulfanilamide or, in combination with oxaloacetic acid and a "condensing enzyme," will produce citrate. In such a reaction, the muscle enzyme is analogous but not identical to phosphotransacetylase which, by contrast, is not inhibited by Na^+ and does not require two molecules of DPN.

A transfer of the acetyl group of acetylphosphate to cysteine can also be accomplished and in the course of measuring the acetyl cysteine (indirectly indicated by the disappearance of acetyl PO_4) the data revealed a phosphatase activity of the enzyme which will not operate without DPN and which can be inhibited by cysteine, glutathione, cyanide (0.001 M), or other compounds which maintain the enzyme in the reduced state.

M.-L. J.

YALE MEDICAL SOCIETY

February 11, 1953

PULMONARY HEART DISEASE. By C. H. Stuart Harris, Professor of Medicine, University of Sheffield, Sheffield, England; Visiting Professor, Albany Medical School, Albany, New York.

Sheffield, with its damp climate and adjacent mining areas, offers an excellent opportunity to study the chronic lung conditions underlying pul-

monary heart disease. This investigation of sixty-four patients from Sheffield and surrounding communities was undertaken in the hope of shedding more light on these underlying lung conditions and the etiology of the seasonal exacerbations that brought the patients into failure.

The sex distribution of these patients, fifty-eight males to eight females, was slightly lower than the 9:1 ratio usually cited for England. The age span, fifty to fifty-nine years, was ten years older than that reported for the United States. The reversible bouts of pulmonary heart failure of these patients had a definite seasonal pattern, the high coming in January and February, and the low coming between May and September.

In fifty-one of these patients, the underlying lung condition was chronic bronchitis and emphysema. In nine others, the underlying lung condition was bronchiectasis. Strangely enough, although Sheffield is surrounded by mining areas, none of these patients gave any indication of an underlying pneumoconiosis. In most of these patients, annual exacerbations occurred with pneumococcal infections marked by cough, sputum and increasing dyspnea. In the course of years, the patients noticed a progressive edema which eventually became the limiting factor of these annual illnesses. The organism isolated in forty-nine out of ninety-four reported exacerbations was the pneumococcus. In spite of the use of antibiotics, patients would return with relapses, usually involving different pneumococcal types.

The pulmonary arterial pressure of these patients when they were in bouts of failure rose to 50 mm. of mercury. However, when they were out of failure, their pressures dropped to normal levels between 20 mm. and 30 mm. of mercury. This reversibility indicated that there was little actual myocardial damage.

Unlike patients with mitral stenosis or mitral insufficiency, who have a low level of serum CO_2 combining power and a normal level of serum O_2 combining power, these patients had a high level of CO_2 combining power and a low level of O_2 combining power in their serums. This condition tended to depress the respiratory center and add to the patients' dyspnea. However, this condition was also reversible, because when the patients were out of failure the serum O_2 returned to normal levels.

In the future, it is planned to investigate the etiology of pulmonary heart disease by a study of patients who are suffering from the early stages of chronic lung disease.

G. McK.

NU SIGMA NU LECTURE

February 11, 1953

CAESAREAN BIRTH AND THE MURLESS HEAD EXTRACTOR. By S. R. Carrabba, St. Francis Hospital, Hartford, Connecticut.

The head extractor described by Murless in 1948 to aid low caesarean section has been utilized for the first time in this country. The instrument is S-shaped and contains a hinge in the middle which locks by means of a sliding sleeve. The single blade resembles the ordinary obstetric forceps blade with an exaggerated cephalic curve. The blade is inserted beneath the fetal head through the lower angle of the uterine incision, the flexed handle obviating interference from the patient's protuberant abdomen. Once the blade is in place, the handle is elevated and locked. With gentle fundal pressure the head may be delivered.

Application of this technique to 40 unselected low sections revealed no significant trauma to mother or baby in any instance. These included some sections before labor but a larger number of patients were operated upon after a trial of labor with such complications as "disproportion," cervical dystocia, and placenta previa (two). The instrument was adapted equally well to oblique transverse positions of the vertex (some deeply impacted). The extractor was also used in two breech presentations. It is suggested that once the operator has learned to use the Murless extractor on easier cases, he may be able to extract the firmly engaged head where manual attempts are sometimes difficult. Advantages claimed include less loss of blood, prevention of uterine tears, good visualization of the field, and extraction of the head in any position without appreciable trauma.

A recent modification of Murless' design has a single lock which closes immediately on elevating the handle, eliminating rough edges and some difficult manipulations encountered in locking the original hinge.

O. L. K.

ANESTHESIOLOGY SEMINAR

February 13, 1953

THE INFLUENCE OF ANESTHESIA, DRUGS, AND TECHNIQUES ON THE CEREBROSPINAL FLUID PRESSURE. By C. R. Stephen, Department of Anesthesiology, Duke University, Durham, North Carolina.

The difficulty in regulating the cerebrospinal fluid pressure during an operation has plagued anesthetists and neurosurgeons for a long time. Whether this difficulty is due to the patient's basic constitution, the use of certain drugs, the surgical manipulations, or the anesthetic techniques is not known. In this series of experiments a catheter attached to an electronic manometer was introduced into the subarachnoid space and a continuous recording was taken from an hour pre-operatively to the termination of the operation, in an attempt to evaluate the factors influencing the cerebrospinal fluid pressure.

Conscious patients showed transient spikes in pressure with voluntary coughing and variations with heaving respiration and normal breathing. Patients subjected to a rough induction period showed rises in pressure up to 900 mm. above normal which tended to persist through the early phases of the operation, sometimes requiring an hour to return to normal. In one patient with a subdural hematoma and a base-line pressure of 450 mm., the induction with ether was extremely stormy. The subsequent coughing and "bucking" pushed the pressure first to 1100 mm. and then to 1900 mm., a level previously considered to be incompatible with life. On the other hand, patients with a smooth induction period often showed little rise in pressure throughout the course of the operation.

From these experiments it is inferred that the rises in pressure are more directly related to the anesthetic techniques than to the anesthetic agents. Surgical manipulations such as pressure on the dura, tilting the patient, and sawing the cranial bone also promote rises in pressure.

Interestingly enough, the administration of Avertin to five patients raised the cerebrospinal fluid pressure 250-300 mm. above normal. This finding contradicts the conventional concept of Avertin as a cerebrospinal fluid depressant.

Also, in these experiments, there appeared to be no consistent relationship between the cerebrospinal fluid pressure and the venous pressure, whereas the more conventional view is that they are very closely allied.

G. McK.

MICROBIOLOGY SEMINAR

February 13, 1953

ENZYMATIC CONTROL OF FIREFLY LUMINESCENCE. By W. D. McElroy, McCollum Pratt Institute, Johns Hopkins University, Baltimore, Maryland.

The significance of firefly luminescence rests in the problem of energy transformation and the mechanism by which phosphate bonds are transferred and coupled. In the presence of oxygen, Mg^{++}, adenosinetriphosphate (ATP), and an enzyme, the firefly will produce a primary luminescent reaction which tapers rapidly and levels at a fraction of the initial flash. If it is postulated that the various components form an active intermediate which may be oxidized to an activated state and produce light or, in the absence of oxygen, form an inactive complex with Mg^{++} and protein, then the base line of luminescence is an equilibrium of active and inactive complex. Under anaerobic conditions the inactive state obtains and the enzyme is immobilized, whereas the effect of oxygen will vary with the concentration of the active intermediate.

The addition of inorganic pyrophosphate and ATP after the initial luminescence will elicit a second flash. However, the pyrophosphates compete with ATP to form inactive intermediates, and the appearance of the second flash corresponds to the hydrolysis of the pyrophosphate. Coenzyme A can also play a rôle in this postulated reaction since, in the presence of a protein factor, coenzyme A will bind the inhibitor in a complex and thereby shift the inactive complex back to an activated state.

M.-L. J.

THE SIXTH EDGAR ALLEN MEMORIAL LECTURE

February 16, 1953

THE APPLICATION OF ORGAN CULTURE TO SOME PHYSIOLOGIC PROBLEMS. By Honor B. Fell, Strangeways Research Laboratory, Cambridge, England.

When organ rudiments or fragments are cultured *in vitro,* they retain or acquire histologic characteristics and sometimes even anatomical structures which are normal for the tissue. Although these explants must be provided with abundant nutriment and oxygen, extensive cellular outgrowth is discouraged by frequent transplants to a fresh medium. Blood plasma as well as embryo and adult tissue extracts are the usual nutrient source if the organs will grow in heterologous medium; some, however, require more homologous preparations. It is noteworthy that tissue cells which have been dispersed by enzymatic digestion of the intercellular cement and suspended in fluid medium will subsequently form cell groups, and these clusters will differentiate when transferred to a semi-solid clot.

Organ culture technique has provided conclusive data concerning the mode of development of such tissues as the salivary glands and pituitary.

It has been used to establish the specific effect of a certain vitamin or hormone on a particular tissue and thereby indicate direct action rather than a mediated mechanism. The effect of insulin on developing bone, for example, has been shown to be hormonal rather than secondary to hypoglycemia. Excess vitamin A in bone culture will result in the disappearance of the matrix and cartilage, whereas in cultures of ectoderm it suppresses keratinization and effects a reversible transition to mucous epithelium. Such applications of this technique indicate unique possibilities which cannot be attained with other tissue culture methods.

M.-L. J.

ANATOMY SEMINAR

February 17, 1953

SOME OBSERVATIONS ON INJURY AND REGENERATION OF MAMMALIAN STRIATED MUSCLE. By Gabriel C. Godman, College of Physicians and Surgeons, Columbia University, New York.

The process of repair in mammalian striated muscle following injury consists of two stages which merge and overlap: an immediate degeneration followed by a period of regeneration and reconstitution. These processes have been observed in samples of muscle obtained from mice infected with Cocksackie virus and in non-infected traumatized muscle from rabbits.

The degenerative processes are divided into five phases:

1. Discoidal splitting with fragmentation in the I bands
2. A proliferation of nuclei and accentuation of the A and I bands
3. A thickening of fibrils, which tend to become more basophilic
4. Mineral precipitation in the form of longitudinal striae
5. Swelling of the broken segment into a brittle pycnotic mass of enhanced birefringence. Reconstitution begins with the contraction of this protoplasmic clot leaving an empty sarcolemmal "hull."

There is initially an accumulation within this "hull" of giant cells, histiocytes, and other detached isolated cells. Some of these "histiocytoid" cells presumably participate in the regeneration which will ultimately take place.

Regeneration then proceeds by either of two methods: (i) The proliferation into the sarcolemmal tube of slender growing sarcoblastic strands, (ii) The proliferation of a giant cell-like bud which completely fills the sheath. The process of redifferentiation which follows resembles embryonic differentiation.

Tissue culture studies of muscle explants demonstrate the growth of redifferentiating sarcoblastic strands similar to those observed *in vivo*. Division of nuclei is amitotic and growth proceeds from the cut stump of the muscle explant. Segments of these strands are seen to fragment into individual cells and assume the morphology and presumedly the function of macrophages. These very same cells then rejoin the growing strands and proceed to manufacture new myofibrils.

It is suggested, therefore, that at least some of the "histiocytoid" cells occupying the sarcolemmal tube *in vivo* arise from the injured muscle itself and that these cells eventually participate in its subsequent regeneration.

E. D. C.

HISTORY OF MEDICINE LECTURE

February 18, 1953

THE FIGHT AGAINST CHOLERA. By L. G. Stevenson, Department of Medical History and Literature, University of Western Ontario, Faculty of Medicine, London, Canada.

There were three pandemics and two intercontinental epidemics of cholera in the nineteenth century, though it probably had a long career in India before this time. These occurred between 1816-1823, 1826-1837, 1846-1863, 1865-1875, and 1884-1892. The first epidemic started in the delta of the Ganges and followed trade routes to Asia and Africa. In 1826 the first pandemic commenced by slow spread up the Ganges, across the Caspian Sea to the Black Sea, up into Moscow by 1830, and simultaneously up the Danube into southern and central Europe and by sea to England. The first case of cholera diagnosed in England was reported in Sunderland in October of 1831.

From England it spread to Scotland and thence to Ireland. The Irish emigrants brought it to Quebec and Montreal, the first North American case of cholera being diagnosed in June 1832. The pandemic then moved down the Lake Champlain region, along the Erie Canal to Albany, thence to New York City and Philadelphia. There is a possibility that some cases were brought directly to New York City from Ireland. Boston reported relatively few cases and ascribed this to the quarantining of vagrants from New York and to a recent sanitation program. It was observed that this disease showed a predilection for areas of poverty and moral decay. The latter observation provided the substance of many sermons for several years following.

John Snow, in St. James's Parish, London, made the first epidemiological spot map, that of the area around the Broad Street pump, to prove the rôle played by contaminated drinking water in the spread of the disease. It is interesting to note that previous to this there are records of ship doctors advising port authorities to have the villagers boil their drinking water when a cholera-ridden ship arrived in port.

In 1883, Robert Koch isolated and demonstrated the vibrio of cholera but could not produce the disease in animals. Max Pettenkofer believed that a gas produced by the organism in damp soil was the cause of cholera. Ferran y Clua actively immunized 50,000 people in Valencia against cholera (1881-85).

An important cholera epidemic occurred in Egypt in 1947 but was finally stopped by public health measures, particularly mass vaccination. It is still endemic in India and other parts of the East. "We have not killed the dragon, but have driven it back into its lair."

J. M. Q.

HISTORY OF MEDICINE LECTURE

February 19, 1953

RATS, LICE, AND IMMIGRANTS: THE STORY OF TYPHUS. By L. G. Stevenson, Department of Medical History and Literature, University of Western Ontario, Faculty of Medicine, London, Canada.

Typhus fever probably entered Europe through Spain from its aboriginal home in the East. In Europe, a syndrome resembling typhus was first mentioned in the twelfth century, and was differentiated from typhoid fever in

1837 by Gerhard in Philadelphia. Nicolle identified lice as the transmitting agent in 1907, and in 1914 von Prowazek described Rickettsiae as the causative organism. Typhus spread throughout Europe in a fifteenth century epidemic, entering England at Oxford, then "lousy," in 1587. The succeeding three centuries, full of rats, lice, and overcrowding, saw waves of highly fatal typhus "affecting the poor in peace, all in war."

Cortez in 1519 brought typhus to North America, where it spread so far as to decimate a French army in the eighteenth century. Apparently forgotten in subsequent years, the disease was reintroduced in Canada by Irish immigrants in the nineteenth century, at a time when there was increasing control of it through hygiene. A typhus pandemic exploded in Serbia in 1915 causing four million deaths by 1921. It has since been endemic in Eastern Europe and in the Near East. The use of DDT in Naples in World War II for the first time aborted an epidemic. An interesting account of this disease can be found in Zinsser's *Rats, Lice, and History*.

MILTON CORN

MICROBIOLOGY SEMINAR

February 20, 1953

CONTROL OF THE METABOLISM OF VIRUS INFECTED BACTERIA. By Seymour Cohen, Department of Pediatrics, University of Pennsylvania Medical School, Philadelphia, Pennsylvania.

Although E. coli strain B is rich in both nucleic acids, T phages infecting this bacterium lack ribonucleic acid (RNA) and appear to inject only desoxyribonucleic acid (DNA). After infection, the synthesis of host material ceases, yielding to the exclusive production of virus—hence DNA. In fact, if the host DNA is labelled, virus pyrimidines can be traced to the host cytosine from which they have been transferred to the phage via desoxyribosides.

The study of the preferential production of DNA leads to a consideration of the two pathways by which E. coli can utilize glucose: the Emden-Meyerhof scheme and the oxidative shunt. Infection with phage will block the pathway which leads to the production of pentose and its incorporation into RNA. This shunting of the metabolism into the production of desoxypentose is not effected by the rapid utilization of DNA in phage synthesis nor by the blockage of any known specific reaction step. Presumably, the incorporation of ribosides into RNA is inhibited. The controlling mechanism is apparently in the virus nucleic acid, since one-third of the total virus DNA is resistant to hydrolytic enzymes and this fraction contains almost all the 5 hydroxy-methyl-cytosine present. This moiety, not present in host DNA, protects the nucleic acid of the phage from degradation by the hydrolytic enzymes of the host.

M.-L. J.

PHARMACOLOGY SEMINAR

February 26, 1953

ANTAGONISTS TO CORTISONE AND ACTH-LIKE ACTION OF STEROIDS. By Robert Gaunt, Endocrine Research, Ciba Pharmaceutical Products, Summit, New Jersey.

A series of compounds were examined for their ability to prevent the adrenocortical atrophy accompanying long-term cortisone therapy. When

given to mice in conjunction with cortisone, a number of steroids proved effective, notably those containing a β-hydroxyl group and an α-methyl group on position 17 and an oxygen linkage on carbon three. Especially potent were methylandrostenediol, dihydrotestosterone, methyl testosterone, and testosterone propionate in the order named; androgenicity was not correlated with ability to prevent adrenocortical atrophy. Although these and other steroids preserved normal size of the gland, it was not shown that they preserved normal function. The probable mechanism underlying adrenocortical weight preservation is in part a direct action of the steroids on the gland and in part a blocking of the ACTH-inhibiting action of cortisone. There was no relationship between the effectiveness of a compound in preventing adrenocortical atrophy and its ability to antagonize the growth-inhibiting property of cortisone.

J. P. G.

PHYSIOLOGY SEMINAR

February 26, 1953

FUNCTIONS OF THERMAL RECEPTORS. By Ingve Zotterman, Royal Veterinary College, Experimentalfältet, Stockholm, Sweden.

The physiological basis for certain psycho-physical phenomena of temperature perception has been quantitatively studied in experiments on thermoreceptors in the tongue. The experiments, done on single warm and cold receptors, show that the differential firing of these thermoreceptors under various temperature conditions correlates with the sensation experienced under the same conditions. For example, if a cold object is placed on an area containing cold receptors, a sensation of cold is immediately felt. If the object is then removed, the feeling of cold is lost, but shortly returns before finally disappearing. By means of an apparatus using running water to control the temperature, cold receptors in the tongue were subjected to a sudden change of temperature analogous to that in the situation described, and the frequency of firing in the fibers was recorded. With the initial sudden temperature drop, the frequency greatly increased but then gradually fell. When the initial temperature was suddenly restored (as the object was removed), the frequency dropped to a subnormal plateau, then rose above the normal frequency and finally sloped to the normal constant rate of firing.

This sensation of paradoxical cold was also demonstrated to be dependent on the physiological behavior of the receptors. Cold fibers were subjected to a gradually increasing temperature from 25.9° C. to 50° C. The frequency of discharge by the fibers increased until nearly 40° C. at which it abruptly dropped to a plateau which persisted up to 45° C. From 45° C. to 50° C. the frequency increased. This phenomenon thus correlates with the sensation of cold which follows a rise in temperature above 45° C.

An interesting corollary to these experiments is that despite the larger size of the warm fibers, the maximum frequency at which their endings fired was considerably lower than that of the cold fibers; the warm fibers fired at four impulses per second, while the cold fibers discharged at ten impulses per second.

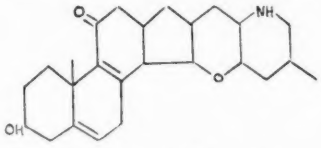
G. A. F.

CHEMISTRY COLLOQUIUM

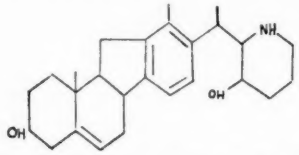
February 27, 1953

STRUCTURE STUDIES ON THE VERATRUM ALKALOID, JERVINE AND VERATRAMINE. By Oskar P. Wintersteiner, Squibb Institute for Medical Research, New York.

Jacobs originally proposed that jervine and veratramine contained a steroid nucleus with an oxygenated carbon 11 which prompted the suggestion that such compounds might be used in the synthesis of cortisone. Subsequent investigators substantiated the A and B rings and the oxygen in position 11. The following formula was therefore suggested for jervine:



Nevertheless, ultraviolet spectrum studies in the current work indicated that jervine was probably not a steroid, and reversal of the C and D rings was suggested. Subsequent structure studies on breakdown products substantiated this hypothesis. Similar experiments with veratramine (the product of hydrolysis of pharmacologically active veratrum esters) led to the conclusion that it, too, had reversed C and D rings. The formula for veratramine, then, would be as follows:



Work in other laboratories showed that the plant steroid, rockogenin, may be converted into a compound with reversed C and D rings, further strengthening the idea that this structure may be a naturally occurring one.

O. L. K.

PLANT SCIENCE SEMINAR

February 27, 1953

THE EFFECT OF ENVIRONMENT ON PLANT GROWTH. By F. W. Went, Department of Botany, California Institute of Technology, Pasadena, California.

The Earhart Research Laboratory of the California Institute of Technology provides 45 combinations of controlled light, temperature, and humidity in which experimental plants can be grown to maturity. There are also facilities for studying the effects of wind, rain, natural and artificial light, and atmospheres of different gas composition. Thus it is possible to simulate certain aspects of climate.

It is well known that in general plants grow best if the temperature during the night is lower than the day temperature. A somewhat unexpected result is that the flowering of certain plants, particularly trees and shrubs,

but even of some grasses and crop plants, is a function of the night temperature as well as of the photoperiod (number of hours of light and dark per day). Potatoes, for another example, when grown at night temperatures over 20° C. do not form tubers, though the tops of the plants look quite usual.

In general, the optimum day temperature for plant development depends on the night temperature. Three-dimensional graphs may be used to pick out optimal growth conditions for a given plant. The method can be extended to include the effects of photoperiod. There is a remarkable correlation between the laboratory-determined, optimal combination of these three factors and the corresponding climatic conditions obtaining in nature where the given plants flourish.

Water relations is another physiological aspect studied. One result shows the importance of dew to some species—the watermelon, for instance. Some plants can not only utilize dew, and grow better with it, but are able to secrete into the soil water taken up as dew, thus storing it, and distributing it to other plants.

Biological specialization in water relations extends even to germinating seeds. Desert plants show some curious properties adapting them to special ecological situations. Some seeds, for example, germinate only if wet from above, as by a heavy rain, and will not sprout if the soil is moistened from below. These seeds contain an inhibiting substance which has to be leached out. Other plants, inhabitants of gullies and washes, bear seeds which germinate only when the impermeable seed coats are ground away; this is done by the agitation of gravel particles in swiftly moving water during heavy rains. All of these mechanisms seem to insure that a seed will only germinate when conditions are such that the resulting plant will be able to reach maturity. It is found that when these plants are grown under crowded conditions, they all reach maturity and produce some seed, though they will all be stunted. This is in contrast to mesophytic plants, which, when crowded, compete to such an extent that only a few reach maturity.

The effects of smog are being studied. The peroxides and ozonides of hydrocarbons are toxic at one part in ten million, a concentration which is approached in regions where smog is severe. Lower concentrations reduce growth and cause lesions on leaves—these may be seen on plants in regions where damage from smog is not specifically acknowledged. It is believed that this decreases the response of the plant to its own growth hormones.

R. R. L. G.

MICROBIOLOGY SEMINAR

February 27, 1953

THE EXTRACELLULAR CULTURE OF AN INTRACELLULAR PARASITE. By William Trager, Rockefeller Institute of Medical Research, New York.

The requirements of *Plasmodium lophurae* are adequately satisfied by an intracellular existence in the duck erythrocyte, and merozoites which are liberated from these cells in the course of normal development will perish unless another red cell is immediately available. However, these parasites can be removed from their host cells by incubation with anti-duck, red-cell serum plus complement and subsequently can be maintained extracellularly for several days.

The plasmodia and red-cell nuclei freed by this type of lysis are suspended in nutrient media containing duck erythrocyte extract. If the preparation is

agitated in small flasks under an atmosphere of 95% air and 5% carbon dioxide, the free parasites adhere to the scum of cellular debris which lines the flask and are thus provided with a semi-solid support in a well-aerated medium. Although degenerate forms can be constantly observed by means of the phase microscope, as well as staining with Giemsa, viable organisms undergo nuclear division and segmentation of daughter parasites. By utilizing this technique, the effect of various compounds on the extracellular survival of *Plasmodium lophurae* can be established. For example, adenosine triphosphate or sodium pyruvate can be shown to reduce the number of degenerate forms, whereas coenzyme A has a delayed, albeit similar, effect, and TPN, no effect at all.

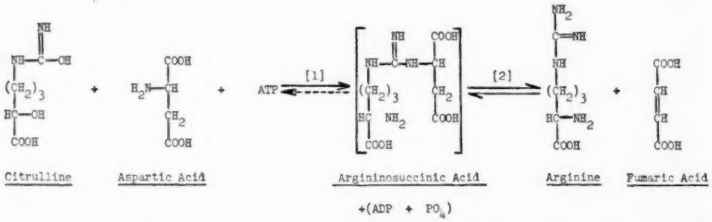
M.-L. J.

BIOCHEMISTRY SEMINAR

March 3, 1953

SOME NEW PHASES OF THE UREA PROBLEM. By Dr. Sarah Ratner, Department of Pharmacology, New York University College of Medicine, New York.

An essential step in the synthesis of urea is the conversion of citrulline to arginine, which is subsequently cleaved by arginase. Two years ago the author demonstrated that arginine is formed from citrulline and aspartic acid through the mediation of ATP. The reaction has now been separated into two stages as follows:



The first step involves the combination of citrulline and aspartic acid to form an intermediate condensation product, argininosuccinic acid, the anhydride of which is formed spontaneously in acid solution, thus permitting its crystallization. This reaction does not appear to be reversible since, following the addition of labelled phosphate to the mixture no labelled ATP is recovered. The enzyme concerned with this step is obtained from acetone dried liver extracts at a purification of about 30-40X.

The second step is an equilibrium between the intermediate, argininosuccinic acid, and the two end products, arginine and fumaric acid. The enzyme which mediates this stage has been recovered from extracts of yeast and purified more than 100X. The procedure necessitated the removal of arginase and fumarase from the enzyme fraction in order that the two end products might be identified.

The reaction is not inhibited by any of the following organic acids: aspatric, glutamis, guanidinosuccinic, succinic. Arginine and fumaric acid do inhibit the reaction, presumedly by shifting the equilibrium to the left.

E. D. C.

YALE MEDICAL SOCIETY

March 4, 1953

CIRCULATORY CHANGES IN MONKEYS DURING ACUTE ANAPHYLAXIS. By Frieda G. Gray and Frank D. Gray, Jr., Department of Medicine, Yale University School of Medicine.

Clarification of the pathogenesis and physiological effects of cardiovascular lesions due to hypersensitivity i sthe goal of a series of experiments in progress. The present report concerns itself with the first study in this series and is designed to reveal the changes in cardiovascular dynamics in acute anaphylaxis.

Three groups of young Rhesus monkeys were used: (i) the experimental group receiving a sensitizing dose of 20 cc. of horse serum, followed three weeks later by a shocking dose of 10 cc. of horse serum, (ii) a control group of non-sensitized monkeys which received a dose of 10 cc. of horse serum only, and (iii) a second control group which received no horse serum, to provide base line studies of cardiovascular dynamics during the manipulations necessary for obtaining and recording the experimental data. Observations were made 15 minutes before, 15 minutes, 30 minutes, and 60 minutes after the intravenous administration of the shocking dose. The techniques used included cardiac catheterization and femoral artery catheterization to obtain pressures and blood samples for blood oxygen saturation, oxygen consumption, and determination of cardiac output. Group (ii) showed moderate rises in right ventricular and femoral artery systolic pressures and oxygen consumption. There was no change or slight increase in systemic blood flow and decrease in circulation time. Group (i) animals showed an average decrease in systemic blood flow of 48%, in femoral artery systolic and diastolic pressure of 48%, and mixed venous blood oxygen saturation of 50%, while an average increase in right ventricular systolic pressure of 93% and circulation time of 62% was observed. It is believed that enough evidence has been presented to demonstrate that tissue anoxia attributable to both perfusion failure and to lowered oxygen tension occurs in acute anaphylaxis in the Rhesus monkey.

J. M. Q.

AN OUTBREAK OF ECTROMELIA IN THE U.S.A. By John J. Trentin, Department of Anatomy, Yale University School of Medicine.

Although Marchal first described ectromelia in 1930, and Burnet subsequently placed it with the pox group of viruses, outbreaks among the mouse populations of this country have not been confirmed. In 1951 a fatal, self-limiting disease spread through a colony of some 10,000 mice bringing death to more than half of the animals. Recognition of visceral lesions characteristic of acute ectromelia and inoculation of infected material into normal mouse foot pads, resulting in either the acute disease or the typical cutaneous lesions of the chronic form, made the diagnosis of this viral malady quite probable. Passage of the agent from infected mouse liver to egg produced the pock-like lesions first described by Burnet, and electron micrographs of these tissues revealed intracytoplasmic, eosinophilic inclusions. Since Burnet had proved, moreover, the immunological cross reactivity between vaccinia and "mouse pox," the diagnosis of ectromelia was definitely established by serological methods which demonstrated vaccinia antihemagglutinins in the sera of convalescent mice and successful cross-immunization with vaccinia.

M.-L. J.

THE BIOLOGICAL SIGNIFICANCE OF FIBROSARCOMA SUSCEPTIBILITY IN MICE. By L. C. Strong, Department of Anatomy, Yale University School of Medicine.

Observations on the latent period of methylcholanthrene-induced fibrosarcoma development in inbred mice have implicated genetic factors in the appearance of these tumors and stimulated speculation regarding the operation of such factors in the development of human tumors. It has been observed, for example, that there is a decreased susceptibility in female mice to methylcholanthrene-induced tumors as the age of the mother increases (between the second and sixth litters). It is usually stated that the current increase in the incidence of cancer in man is directly correlated with the increase in life expectancy which has occurred during the last fifty years. Nevertheless, should man resemble the mouse, it is evident that his susceptibility to tumor development is probably a more complicated problem than mere life expectancy. Life expectancy itself has been shown to be influenced by parental factors in man. Increased age of the parents at death increases the life expectancy for the children. Life expectancy in the offspring is reduced as the age of the mother increases at delivery (but is not influenced by the age of the father). It is suggested, moreover, that the increased incidence of cancer in man may be influenced by the reduction in family size in modern times. Since mice show a decreased susceptibility in the later offspring, the average susceptibility is lower in members of large families than if, as in the modern family, no more offspring are produced after the first, more susceptible.

The analysis of the human population is probably complicated further by the fact that susceptibility as a biological characteristic is best expressed during the age of maximum sexual activity and declines in adolescence or senescence. This phenomenon was observed in mice and indicated that age should be considered in cancer studies in man.

O. L. K.

MICROBIOLOGY SEMINAR

March 6, 1953

TISSUE LEVELS OF ADENINE NUCLEOTIDES. By Harry Albaum, Department of Biology, Brooklyn College, Brooklyn, New York.

By the Cohn and Carter method for the analysis of adenine nucleotides, it can be shown that preservation of ATP in tissues at —18° C. is quite variable, since muscle ATP remains unchanged in a one-month period, whereas kidney and liver ATP is only moderately stable and brain ATP disappears altogether. A similar differential stability in these tissues was found when anoxia was produced by CN^-. The apparent greater stability of ATP in muscle can probably be ascribed to partial restoration of ATP and phosphocreatine by glycolysis, while in brain tissue, where ATP can only be produced by aerobic phosphorylation, it disappears completely. If anoxia is induced by N_2 breathing, the different centers of the brain show varying ATP deficiencies directly related to impaired function. Immediate exposure to high altitude conditions had comparable effects but, if test animals were adjusted to such conditions by a gradual reduction in pressure, ATP levels were maintained. Apparently compensatory vascularity prevented anoxia and consequent reduction of the nucleotide.

Some related studies of ATP production have shown that inorganic P is rapidly incorporated into ATP in the presence of malignant growth and

during pregnancy. Intramuscular injections of adenylic acid will augment the blood level of ATP in excess of the amount of adenylic acid injected, indicating a mobilization of some labile reservoir; if the adenylic acid is tagged with labelled P, the highest concentration will be found in the adrenals.

M.-L. J.

THE BEAUMONT CLUB

March 6, 1953

CHEMICAL WARFARE MEDICINE IN TWO WARS. By L. L. Waters, Department of Pathology, Yale University School of Medicine.

Many advances have been made in chemical warfare since the Greeks first blended pitch, sulfur, oil, and other ingredients into suffocating incendiary bombs which were hurled onto enemy ramparts or decks of opposing war galleys. In 1899, at the Hague, the principal European countries signed a treaty outlawing chemical warfare.

In 1912, in France, tear gas grenades were first used in dealing with civil disturbances. It was, however, the Germans who initiated gas warfare on April 22, 1915, "the black day of Ypres," by the release of a chlorine cloud against a five-mile front of Canadian and French Colonial troops; 5,000 casualties resulted and the line, unbeknownst to the Germans, was thrown into panic and broken. It was not until September of that year that the Allies were able to reply in kind. In December, 1915 the Germans introduced phosgene in cloud form. Its chief pathological effect, like that of chlorine, is a rapidly developing, massive pulmonary edema. In 1916 both sides began using phosgene as well as di-phosgene-filled artillery shells.

On July 12, 1917, again near Ypres, the Germans introduced mustard gas ("Yperite"). This was the first use of the vesicant or blister gases. A year passed before the Allies were able to retaliate with mustard-filled artillery shells.

By the end of the war, all nations were using the lung irritants, the vesicant mustards, and various vomiting and tear gases. Lewisite, an arsenical vesicant, was ready but not used.

The Geneva gas protocol of 1925 outlawed gas warfare and was subscribed to by thirty-nine countries. However, the treaty of 1923 with Guatemala, Salvador, Honduras, and Costa Rica is the only one binding the U.S.A. not to engage in chemical warfare.

In England, at the Experimental Station of the Royal Engineers at Porton, Joseph Barcroft headed a group to study the toxicity and physiology of asphyxiating gases. German researches along similar lines were published in the *Zschr. ges. exp. Med.* in 1921. France sponsored the development of a nitrogen homologue of mustard called "French" or "nitrogen" mustard. In 1917 a young Dutchman in an obscure Utrecht thesis recorded the necrotizing effect of mustard gas on the lymph nodes of experimental animals. In the U.S.A. the Chemical Warfare Service of the Army with its Medical Division was established, in July 1918, at Edgewood Arsenal, Maryland.

On October 24, 1919 an explosion in the Breloh munitions dump on the Lüneburg Heath in Germany scattered approximately 50,000 tons of assorted vesicants over the countryside. Dr. Herman Büscher, recently of the German Army Medical Corps, found himself with the largest practice

in gas warfare medicine that any single individual has ever had. His observations were published in 1931 in book form, *Green and Yellow Cross,* and bring to light many clinical observations.

Interest in chemical warfare had generally waned until 1939-40, when a sudden resurgence was experienced. England created the Chemical Defences Research Department, a group which demonstrated the effectiveness of 2, 3 dimercaptopropanol (British Anti-Lewisite, BAL) as an antidote to Lewisite.

In the U.S.A., organization for medical research in relation to chemical warfare was not initiated until 1941. A modern research laboratory was formally opened in the spring of 1944 at Edgewood Arsenal, Maryland. The only phosgene patients studied during the war were those accidentally gassed in the chemical munitions plant at Edgewood Arsenal.

In 1939, a Russian, Porembskii, demonstrated that Hexamethylenetetramine (Urotropin) taken *in vivo* would protect mice against lethal doses of phosgene. These observations were extended in this country to larger animals and similar results recorded. Unfortunately, this compound is of no therapeutic value if administered after the phosgene has come in contact with the pulmonary alveoli.

C. C. Lushbaugh, a pathologist at the University of Chicago, rediscovered that absorption of mustard homologues—nitrogen or sulfur—results in enormous destruction of lymphoid tissue throughout the body. Goodman, Gilman, and Dougherty, with the coöperation of Lindskog at Yale, treated several advanced cases of lymphosarcoma with these agents and noted phenomenal decreases in the size of the tumor masses. A conference held in New Haven in July, 1944, marked the formal launching of the nitrogen mustards into civilian medicine.

During the war, intelligence reports indicated that the Germans were preparing "nerve" gases for possible use. Di-isopropylfluorophosphate, one of the first studied, was not employed as a nerve gas but is now being used therapeutically in the treatment of glaucoma and myasthenia gravis. It produces complete inactivation of tissue cholinesterase. Observation of the actions of fluoroacetates on the myocardium constitutes another significant development in chemical warfare research. One of these compounds, Compound 1080, has enjoyed considerable success as a rodenticide.

Realization of the importance of chemical warfare medical research has resulted in the establishment of the Chemical-Biological Coordination Center at the National Research Council in Washington to assemble and make this information available to scientists the world over.

J. M. Q.

FRANÇOIS QUESNAY; SURGEON AND PHYSIOCRAT. By Samuel C. Harvey, Department of the History of Medicine, Yale University School of Medicine.

François Quesnay, organizer and leader of the Physiocrats, was first a barber-surgeon, later an historian, and finally an economist. He received his M.A. at the University of Paris and was a practising surgeon before the age of twenty. However, it was not until 1744 that he received his M.D. degree at the small university at Pont-au-Mousson, over the protest of the physicians of the University of Paris, who thought that they alone should award this degree. Part of this protest was undoubtedly due to personal feelings

against Quesnay, but it also reflected the changing status of surgery in France. The surgeons had separated from the barbers and formed a college of their own in Paris, much to the distaste of the Paris physicians. In 1737, the French Academy of Surgeons was created, with Quesnay filling the post of perpetual secretary.

During this period, he was not only a busy surgeon, but also an author and an historian. He published treatises on bleeding, gangrene, and suppuration, as well as a two-volume history of French surgery.

Quesnay then turned from the surgery of Paris to the politics of the court of Louis XV. At Versailles, he assembled a group of economists that became known as the Physiocrats. This group, deriving their ideas from the economic changes occurring in England, believed in economy by natural law, or as Gournay, a prominent Physiocrat, put it: "Laissez faire, laissez passer." Besides Quesnay, who was undoubtedly the leader of this group, and Gournay, the Physiocrats included among their members, Turgot, who established Lavoisier and later became comptroller-general of France under Louis XVI, and Mirabeau, a noted orator who was later president of the Jacobin Club and a member of the French National Assembly.

Most of the Physiocrats died before the French Revolution, but the youngest member, Pierre Samuel DuPont de Nemours, narrowly missed the guillotine. He fled from France with his son, Eleuthère Irénée DuPont, who had been learning the powder trade from Lavoisier. It is interesting to note that Irénée DuPont, at the instigation of Jefferson, started in Delaware a powder plant that has since undergone a considerable expansion!

G. McK.

PHARMACOLOGY SEMINAR

March 12, 1953

BLOOD VOLUME: EVALUATION AND INTERPRETATION OF RESULTS. By Dr. Magnus Gregersen, Department of Physiology, Columbia University, College of Physicians and Surgeons, New York.

The accuracy and, hence, the value of blood volume determination by the dye technique (T 1824) as a clinical and investigative tool has often been questioned. This is the result of the discrepancies regularly reported by a number of investigators employing a variety of methods. Values which have been obtained with either radioactive phosphorus or with plasma volume determinations differ significantly. This confusion may be attributed to the variety of techniques devised and the failure adequately to consider sources of error and to employ suitable correction factors. Intravascular hemolysis and the variations in distribution of blood cells with resultant variation in hematocrit will influence the accuracy of results. For example: the value obtained for a true blood volume, i.e., measured cell volume plus plasma volume, when calculated by means of the dye method using T 1824 is observed to increase with an increase in hematocrit. However, the value obtained when calculated by the P^{32} method is lower than normal by a constant factor, irrespective of variations in the hematocrit.

It follows then that for the T 1824 method a suitable correction curve may be constructed, while for the P^{32} method one may abstract a constant correction factor. With sufficient care suitable curves may be drawn for

each method employing standardized techniques to allow calculation of the true blood volume from the experimental result, thus bringing order to an otherwise rather equivocal situation.

E. D. C.

ATYPICAL GROWTH RESEARCH UNIT SEMINAR

March 16, 1953

THE MORPHOGENESIS OF COLLAGEN. By Keith R. Porter, Rockefeller Institute for Medical Research, New York City.

The electron microscope was used in investigating the cellular locus of collagen formation in fibroblasts taken from the dermis of chick embryos and grown in tissue cultures. By this means it was determined that the process is initiated at the cell membrane rather than within the cell. Photomicrographs of the membrane show what seem to be developmental stages from undifferentiated fibrils to fibrils with micro- and macro-striations characteristic of collagen. Photomicrographs of cross-sections reveal fibrils immediately external to the fibroblastic membrane but none deep to it. Complete formation of fibrils, however, does not take place at the cell surface. Some of the procollagen secreted by the cellular membrane is released into the culture medium where it polymerizes into fibrils, the diameter of which increases as more procollagen is added.

Photomicrographs of interstitial spaces demonstrate uncrossed collagen fibrils and fibers. That there is no interweaving of the elements as in a fibrin clot indicates that the fibrils are not laid down simultaneously, but successively.

In contrast to the usual collagen fibrils whose macro-periodicity is 640Å, some fibrils grown in Locke-boullion solution have a striated periodicity of only 220μ. Other fibrils thought to be mucoprotein, and composed of fibers having a periodicity of 1200μ, have been observed to join those of smaller periodicity. It may be that the large fibrils impose on the smaller the periodicity of 650Å characteristic of collagen fibers.

G. A. F.

BOOK REVIEWS

Paging the Polyhistor

TALBOT H. WATERMAN

A review of L. H. Hyman's The Invertebrates. Vol. I, 1940, Protozoa through Ctenophora, xii + 726 pp., 221 figs.; Vol. II, 1951, Platyhelminthes and Rhynchocoela, vii + 550 pp., 208 figs.; Vol. III, 1951, Acanthocephala, Aschelminthes and Entoprocta, vii + 572 pp., 223 figs., New York, McGraw-Hill.

So strong are the centrifugal and fractionating forces in modern science that one may well wonder whether, even by loose analogy, Newton's third law has any validity here—whether in fact there are any effective agents for unity, synthesis, or broad evaluation working in reaction to the primary but usually peripheral quest for the frontiers. The very organization of research and teaching tends to encourage or even enforce microcosmic specialization. Little wonder then that one may often be embarrassed and even alarmed at the aptness of the lampoon-cartoon of the scientist as a man learning more and more about less and less. This aphoristic extreme obviously abets the germination of what William Morton Wheeler[1] so cogently described as academic dry-rot.

The reason commonly given nowadays for this state of affairs is that knowledge is so vast and progress so swift that one man just cannot cope with them. Even an intermediate textbook often must needs be written by many specialist-authors. Yet the integration and assessment of broad fields of learning *has* been accomplished on a grand scale by a few outstanding scholars. Consider Conrad Gesner's heroic *Historia Animalium*[2] organizing, illustrating, and evaluating all of zoological knowledge as it stood in the middle of the sixteenth century. But this cornerstone of modern zoology with its 4,500 folio pages and hundreds of woodcuts was a mere fraction of the published output of this Swiss M.D. who died of the plague before he was fifty!

Such feats seem superhuman and from this very challenge should provoke the scholar as Everest's unscaled tower, the mountaineer. Yet few essay these intellectual heights. Clearly the reasons for this are indeed complex. Whatever they have been in the past, or may be now, the polyhistor is a rare bird.

But rarities do occur. Such a one is Dr. Libbie Henrietta Hyman, whose current project is to collate mid-twentieth century knowledge of invertebrate zoology. With the nearly concomitant publication of the second and third volumes of this treatise the magnitude of the whole undertaking becomes clearer. Already there are 1,800 pages of text, 4,000 or more illustrations, and 150 pages of bibliography. The author's present estimates suggest at least three more volumes will be required to complete the job. If one were not qualmish about certain unfair overtones, he could paraphrase Cuvier's remark that Gesner was the German Pliny with the quip that Hyman is the American Gesner!

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[1] Wheeler, W. M.: The dry-rot of our academic biology. Science, 1923, *57*, 61.

[2] Gesner, Conrad: *Historia animalium*. . . . Froschouerus, Zurich, 1551-87.

On a more critical level it is pertinent to inquire whether Miss Hyman's labors merely reflect her temerity and tenacity or whether they do indeed contribute something important to science and the scientist. The answer is unequivocal, for here is a fine example of the important but uncommon centripetal force discussed above. When one biologist of competence and knowledge critically examines the present state of so broad a field, many interrelationships become clarified as they rarely can by other means. Clearly, the joint efforts of many scientists can produce more in the same time, and the specialist-collaborator can write about his own bailiwick more authoritatively than anyone else. Yet the mere aggregation of such vignettes, however well arranged and edited, can but leave the reader himself to synthesize and evaluate the whole field.

This is not to gainsay the tremendous usefulness and scope of Bronn's *Tierreich*[3] or the Kukenthal-Krumbach *Handbuch*.[4] It is merely to point out that these great collaborative enterprises have certain inherent shortcomings that the work of a single author may overcome. Furthermore, these particular monuments of German zoological scholarship are incomplete and in part far out of date. Grasse's *Traite*[5] currently being issued is an up-to-date French work of somewhat comparable organization.

The plan and scope of the two most recent volumes of Hyman's *The Invertebrates* are closely similar to the first volume *Protozoa through ctenophora* published eleven years earlier. Volume II begins with a fifty-page general introduction to the bilaterally symmetrical animals here treated for the first time in the work. It is such passages as these that best exemplify the rewards of the polyhistoric approach. For here the author can discuss general problems of organization, segmentation, coelom formation, and the like from a panoramic viewpoint unobstructed by local bias. As in the three general chapters in Volume I, the reader is treated to an advanced discussion of zoological fundamentals in refreshing contrast to the textbook clichés usually provided on such matters. Praiseworthy, too, is Miss Hyman's habit in these general chapters, as well as in the rest of the treatise, of clearly presenting the evidence and reasoning leading her to specific conclusions. Naturally, one may differ with the author in her conclusions but he can, nevertheless, see exactly wherein the difference lies. In a day when an almost unreadable string of bibliographical reference numbers, devoid of critical evaluation, frequently passes for an adequate review, this is a rare quality indeed.

Succeeding chapters of Volume II deal with the acoelomate Bilateria: the flatworms (406 pp.) and the nemertine worms (73 pp.). Volume III continues with the pseudocoelomate Bilateria, here divided into three phyla: Acanthocephala (52 pp.), Aschelminthes (467 pp.), and Entoprocta (33 pp.). In each phylum the material is organized on a plan essentially as follows: historical introduction; general characteristics; morphology and life cycles, first for the whole group, then for the sub-groups; embryology; ecology; physiology; behavior; phylogeny; bibliography. Experience has shown that in using *The Invertebrates* the reader may have to familiarize himself thoroughly with the work to make effective use of its assets. Thus,

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[3] Bronn, H. G.: *Klassen und Ordnungen des Tierreichs*. Leipzig, Winter'sche Verlagshandlung, 1859-.

[4] Kukenthal, W. and Krumbach, T.: *Handbuch der Zoologie*. Berlin, Walter de Gruyter, 1923-.

[5] Grasse, P. P.: *Traite de zoologie*. . . . . Paris, Masson, 1948-.

if one were interested in looking up the Turbellaria, pertinent material would be found in the general sections of Volumes I and II, under Platyhelminthes, under Turbellaria, under the turbellarian orders, and *passim* in the phylogeny section. Fortunately, the indexing is good so that at least the mechanical aspects of the task are minimized.

In a work with the breadth of *The Invertebrates* there are bound to be a number of controversial issues raised. Some of these will be due to Dr. Hyman's forthright way of jettisoning well-intrenched, but in her considered judgment, erroneous or misleading concepts. Few of us like to see one of our well-ingrained prejudices exposed as such! Other controversial points result from the incompleteness of our knowledge coupled with the temptation or even necessity of forming a critical opinion. Several of the most general of such issues will be mentioned here.

Basic to her whole concept of the relationships between the phyla of animals is Miss Hyman's faith in embryonic similarities. Of course, she eschews the extreme form of this metaphysics epitomized by the old enthusiasts for the germ layer theory or Haeckel's biogenetic law. Yet her major groupings of animals are defined by their mode or site of blastopore formation and the embryonic origins of their body cavities. However, Miss Hyman herself cautions against the pitfalls of interpreting phylogenetic relationships on the basis of larval stages (Vol. II, pp. 17-18). In the coelenterates she is also quite familiar with equally pertinent caveats. Take, for example, the independent evolution of polyp and medusa in Hydrozoa which frequently makes it impossible to pair up those of the same species except by actually observing one develop from the other, or consider the remarkably varied methods of gastrulation by ingression, by delamination, by invagination which are known to occur in this same phylum even among closely related forms. The current conservative views of embryologists on the significance of the germ layers and on recapitulation are likewise to the point.[6]

All of these lines of evidence lead to the same conclusion, namely, that at all stages of its development and life history an organism must effectively maintain itself in its given environment. Exactly how it does so is unequivocally a result not merely of its phylogenetic history, but of its immediate specific needs and means for living then and there. The solution of this dilemma obviously presupposes a knowledge of that which we are trying to learn.

Further matters of issue revolve around the names and inclusions of the various phyla of animals. The Phylum Rhynchocoela is so named by Miss Hyman to memorialize Schultze's important contribution to our knowledge of this group despite the fact that earlier as well as later workers almost without exception used Nemertina, or closely similar names for the phylum. Another questionable practice which has been used on the Coelenterata (= Cnidaria) and Nemathelminthes (= Aschelminthes) is the one of renaming phyla which have not been identically delimited by various workers in the field. Justification for the new name is claimed on grounds that a change in definition makes the old name ambiguous and hence undesirable. Multiplication of names for such reasons is certainly not condoned in taxonomy where, for genera and species at least, the original

[6] de Beer, G. R.: The differentiation of neural crest cells into visceral cartilages and odontoblasts in Amblystoma, and a re-examination of the germ layer theory. Proc. R. Soc. Lond., 1947, *134B*, 377.

names are frequently expanded or contracted in their coverage as knowledge and understanding increase. The defining of genera and species is a relatively orderly and legitimate business, but even here internecine controversy may rage.[7] How much more difficult is the extra-legal status of the higher taxonomic categories which seem subject to polemics rather than arbitration!

An item that lies more deeply than the question of names also arises in relation to the Aschelminthes, namely, the concept of a phylum. For the assemblage of forms here included certainly is a less compact integral unit than most other phyla. In fact, many zoologists will be tempted to brand such a category, which *inter alia* bonds rotifers, gastrotrichs, and nematodes into one phylum, as a wastebasket. The author was well aware of this likelihood but, nevertheless, like Jenny, she *did* make up her mind.

A large and important part of *The Invertebrates* is its illustrations which fortunately are numerous and informative. One might wish for more technical polish in their execution, but as a whole they do make their points effectively. More serious is the complete failure to show any scale on the drawings. The author may retort that anyone with common sense knows that a whole animal is bigger than its egg and that an organ is somewhere in between. But this hardly seems enough when everything from a sperm to a habit sketch may be assembled in a single plate. Furthermore, in figures taken from other sources one needs to know whether they are used directly, redrawn, or otherwise modified. This is not indicated.

Figures almost never are used to illustrate any points except those dealing with anatomy, histology, and embryology. The nearly complete neglect of graphic illustration in presenting data of life cycles, distribution, physiology, and behavior give the books a superficially old-fashioned look, an appearance, as indicated above, not in accord with the broad scope of textual coverage.

From what has been said it is obvious that *The Invertebrates* is an indispensable work for all zoologists. An even broader usefulness is indicated for the two new volumes here reviewed since many animals of great medical, parasitological, and economic importance are included in the phyla covered.

Biological scientists in general owe a debt of gratitude to Miss Hyman for her sound and stimulating scholarship. Her further progress in completing the whole work will be awaited with great expectation.

THE POSTURAL DEVELOPMENT OF INFANT CHIMPANZEES. By Austin H. Riesen and Elaine F. Kinder. New Haven, Yale University Press, 1952. xii + 240 pp. $5.00.

This monograph is an outgrowth of the studies of physical, psychological, and behavioral development in chimpanzees being carried on at the Yerkes Laboratories of Primate Biology in Orange Park, Florida. It is the first publication of a larger investigation of which subsequent publications will deal with tests of prehensory, perceptual, and adaptive behavior. The present study was the first completed, because postural adjustment is considered to be fundamental to almost every other form of behavior. Actually, the book is not just a study of the chimpanzee but rather a study of the postural development of the chimpanzee during the first year of life as

[7] d'Almeida, R. F. F. and Oiticica, J.: The International Commission on Zoological Nomenclature and the name of the monarch butterfly. Science, 1951, *113*, 728.

compared to man. Only two chapters deal exclusively with chimpanzee behavior. Also, the test procedures employed were part of the Gesell and Thompson behavioral examination; a test devised for the study of the human infant. The most significant findings of this comparative study are that, in general, body postures appear on the average one-third earlier in the chimpanzee, and that chimpanzee postural development is little influenced by environmental variations. Chapter six, which comprises over half the book, is too technical to be of interest to the general reader. However, the study as a whole is a much needed contribution to comparative psychology and should be of great value to those interested in behavioral ontogeny. Students of human child development should also find this study of great interest, for the value of many items of the Gesell examination as indices of development are questioned.

E. S. CRELIN

BLOOD CLOTTING AND ALLIED PROBLEMS. Transactions of the Fifth Conference, January 21-22, 1952. Joseph E. Flynn, Ed. New York, Josiah Macy, Jr. Foundation, 1952. 368 pp. $4.95.

The final Macy conference on blood clotting follows the pattern set by its predecessors. Authorities in different disciplines assembled to discuss various aspects of blood coagulation. At the Fifth Conference the topics of discussion included the clinical evaluation of anticoagulants, new clotting factors, irradiation and hemostasis, and fibrinolysin.

The breadth of the conference was even greater than indicated by the table of contents, for during the presentation of papers the participants interrupted at will, often to make comments and present data tangential to the subject at hand. The comments are often incisive and constructively critical, and many are of more interest than the original papers. The verbatim report is rambling and provocative, probably the most interesting of all the Transactions on blood clotting. It contains a cumulative index to the five volumes which, collectively, are a monument to the advances that have been made in blood-clotting research during the past decade.

Of some interest is the inclusion of a report of a subcommittee in which is listed the synonymy of the factors reported to be involved in the conversion of prothrombin to thrombin. It is hoped that the report is not merely the prelude to an attempt to dictate conformity to a nomenclature approved and recommended by a committee, however enlightened its members; premature standardization could further confuse an already confusing problem.

J. P. G.

COLLEGIATE EDUCATION FOR NURSING. By Margaret Bridgman. New York, Russell Sage Foundation, 1953. 205 pp. $2.50.

Dr. Margaret Bridgman, formerly Dean of Skidmore College, has spent the past two years, under the auspices of the Russell Sage Foundation, in a study of nursing education. This book is the report of her study, with recommendations for the amelioration of the present confused conditions in this field.

At its annual conference in 1951 the Department of Higher Education of the National Education Association passed a resolution emphasizing the need for colleges and universities to assume responsibilities for nursing

education. Dr. Bridgman clarifies the nature of that responsibility. She gives the reasons for the present critical deficiencies in nursing service, pointing out why the demand has increased so markedly and why the supply falls far short of the needs of the people, even though there are more nurses active than ever before. An historical review of the development of nursing education explains why schools of nursing have, until recently, been established in hospitals rather than in educational institutions. Even now, of approximately 1,100 schools of nursing in the United States, only ten per cent have "degree" programs administered and controlled by colleges and universities. The author gives full credit to the leaders of nursing education who have had the vision and the strength to foster the development of the collegiate school and to those institutions of higher learning which have recognized the rights of nurses to an education on a par with other professional workers. On the other hand, she does not spare criticism of those institutions which have considered nursing as an opportunistic sideline.

Reference is made to the wide range and variety of essential functions all classified under "nursing" and to the variety of educational patterns necessary to prepare for those functions. The special plea of this book is for higher standards of education in the established institutions of higher learning, to prepare the well-qualified professional nurse, whose responsibilities for the maintenance of health and for the care of the physically and emotionally ill are far greater than is realized by those not intimately concerned with the problem.

Dr. Bridgman presents a challenge to higher education in no uncertain terms: ". . . it seems undeniable that failure of the educational system to provide opportunities and maintain standards for nursing education is a crucial deterrent to an adequate supply of qualified nursing personnel and therefore to the maintenance of health services at the level demanded by our stage of social development." One may hope that this timely book will be studied by all those concerned with education and with the health needs of the nation.

ELIZABETH S. BIXLER

THE CONTRIBUTION OF MEDICINE TO OUR IDEA OF THE MIND. By Sir Russell Brain. The Rede Lecture for 1952. Cambridge, Cambridge University Press, 1952. 30 pp. $.50.

Sir Russell Brain, President of the Royal College of Physicians and Chairman of the recent International Congress for Internal Medicine, is one of the most able neurologists in the English-speaking world, and anything that he might write on the relation of mind to the structures of the brain will be considered with the greatest seriousness. His Rede Lecture is illuminating, but it does not greatly advance our conception of the mind-body relationship. He, by indirection, confesses the defeat that Sir Charles Sherrington confessed both in his Rede Lecture and in the second edition of his *Integrative action of the nervous system* published in 1947. Nevertheless Sir Russell concludes, "Medicine is also an art, and as an art it has never lost sight of the whole man, the whole that is somehow the sum of its parts. We have never needed that vision more than we do today, when, if man is to survive at all, he must somehow find a way of harmonizing logic, philosophy and humanity."

JOHN F. FULTON

BIOLOGICAL CHEMISTRY. By Alexander Gero. New York, The Blakiston Company, 1952. xvi + 340 pp. $5.00.

This book is proposed to serve as a nexus between undergraduate organic chemistry and graduate or medical school biochemistry. An indication of its scope can be gained by a listing of the table of contents. There are two chapters on physical chemistry, single chapters on the electronic theory of organic chemistry, optical isomerism, protein chemistry, carbohydrate chemistry, lipid chemistry, steroids, isoprenoid compounds, aromatic compounds, heterocycles, biochemical reactions, chemical structure and physiological activity, research methods, syntheses of important compounds, and a final list and description of recommended textbooks and monographs for supplementary reading. The book is thus comprehensive. But it lacks depth; this is especially marked in the presentation of physical chemistry and the discussion of chemical structure and physiological activity. The author states in his preface that "this text is essentially a somewhat advanced organic chemistry." Although the book may suffer from this pretense, it does not suffer from such substance.

J. P. G.

ATLAS OF SPATIAL VECTOR ELECTROCARDIOGRAPHY. By J. W. Hurst and G. C. Woodson. New York, Blakiston Co., Inc., 1952. 207 pp., 92 figs. $6.00.

This monograph is similar physically and in content to Grant and Estes' monograph of the same title, published in 1951. In the words of the authors, "it is an extension of the . . . material to include the clinical application of the vector method."

In the first chapter a simple method of derivation of vectors from the standard leads of the electrocardiogram is offered. All steps are well illustrated by diagrams and the analogous use of familiar objects. Due attention is paid to the basic "ground rules" to be observed in the vector representation on a hexaxial reference system. For the sake of brevity and clarity, at the lesser risk of being dogmatic, the authors have avoided much theoretical background.

Having mastered the method itself, the reader will approach the latter sections of the book with interest. Here electrocardiograms quite typical (i.e., by pattern) of a variety of electrocardiographic syndromes are subjected to vector analysis. Many references are made to clinical and anatomical data in the interpretation of the final spatial vector arrangement.

The monograph serves as an excellent introduction to the vector method which is of established value as a teaching tool. On the basis of this presentation, however, it is difficult to appreciate how this method offers a "more basic approach to electrocardiography," or avoids a great deal of memorization.

This book should prove of interest and of value to the student first embracing the study of electrocardiography and, indeed, for all seeking an introduction to clinical vector analysis.

KENNETH G. JOHNSON

A TEXTBOOK OF HISTOLOGY. By H. E. Jordan. 9th Ed. New York, Appleton-Century-Crofts, Inc. 687 pp., 609 figs. $9.00.

The first edition of this textbook appeared in 1916 and has now undergone its ninth revision. The subject matter is taken up in the conventional

order: protoplasm, the cell, the tissues, and the organ systems (including the special sense organs). It is relatively short, easy to read, and profusely illustrated, but compared with its rivals, rather poorly illustrated. Even though it has more figures than two leading histology texts, these are frequently poorly selected and poorly reproduced.

In his preface the author writes: "An effort has been made in this edition to incorporate important advances during recent years, including certain results of tissue culture and electron microscopy." The extent of this effort is indicated in the bibliography of 514 references of which only 26 titles (about 5 per cent) have been published since 1940, the date of the previous edition. Since then, the principal advances in histology have been the elucidation of the fine structure of the cell and its products by phase-contrast and electron microscopy and the correlation of function with morphology by the techniques of histochemistry, differential centrifugation, and microspectrophotometry. The reader will find little inkling of these in this text. Here the nucleus is still the center of oxidative processes in the cell, the functions of the mitochondria are only vaguely adumbrated, and the Golgi apparatus still holds sway with no suggestion that it may be an artifact. Little or no mention is made of histochemical studies or the chemical significance of the structures and staining reactions described. In an era when many elegant electron micrographs of cells and their products are available, only three are included in this book. The description of the neurohypophysis is completely outmoded. Many pages are devoted to illustration and discussion of old theories and opinions which have been discarded as no longer valid, such as the contraction nodes or waves in sections of fixed smooth muscle (illustrated by seven figures). The pre-World War I classification of glia cells is still preserved. The only important recent advance which has been incorporated in the text is the revised concept of hepatic structure set forth by Elias. In general this book suffers from too close adherence to the content of the previous editions.

SANFORD L. PALAY

MAN AGAINST CANCER. By I. Berenblum. Baltimore, The Johns Hopkins Press, 1952. 170 pp. $3.00.

This well-written, small volume deals broadly with trends in cancer research as well as clinical approaches to the problem. The first section of the book has to do with similarities of tumor cells to the normal tissues from which they arise, and presents a discussion of the genetic factors in cancer. This is followed by a brief introduction to the rôle of radiotherapy and surgery in the treatment of the disease. A short historical review introduces the section on research and is followed by chapters on genetic studies, carcinogens, viruses and cancer, and the potential application of the extensive advances in biochemical techniques to the problem. Some of the difficulties in the development of chemotherapeutic agents are also pointed out. Although intended primarily for the educated layman, this book could be read profitably by medical students and physicians as well as workers in the research aspect of oncology.

HARRIS BUSCH

THREE VESALIAN ESSAYS TO ACCOMPANY THE "ICONES ANATOMICAE" OF 1934. By S. W. Lambert, W. Wiegand, and W. M. Ivins, Jr. (Issued under the auspices of the Library of the New York Academy of Medicine.) New York, The Macmillan Company, 1952. xi + 128 pp. $6.00.

This well-bound volume, which carries a foreword by the Librarian of the Academy, Dr. Archibald Malloch, contains a brief piece by the Vesalian scholar, Samuel W. Lambert (which was intended as an introduction to the *Icones Anatomicae** issued by the New York Academy of Medicine in 1934), a chapter by Mr. Wiegand on the Vesalian woodblocks, and, finally, a longer note by Mr. Ivins, formerly Curator of Prints at the Metropolitan Museum of New York.

The original plates used for the "Icones" were discovered by Mr. Leonard Leopold Mackall in Munich in a library basement in the early thirties; Mr. Mackall reported his discovery to the Academy, and authorization was secured for having the illustrations printed from the original blocks under the supervision of the scholarly printer of the Bremer Press, Mr. Willy Wiegand of Munich. Mr. Wiegand's illuminating marginal notes had been intended, like Dr. Lambert's introduction, for inclusion with the great folio. Unfortunately, the "Icones" appeared without textual explanation. It is, however, gratifying to have these two explanatory essays now to be used in conjunction with the book of plates. Dr. Lambert deals with the amusing initial letters found in the "Fabrica," and Mr. Wiegand describes the plates and explains how it was possible to use them after some four hundred years.

The third essay by William M. Ivins, Jr., has been reviewed in many journals and I find it difficult to make appropriate comment about it because it represents the kind of special pleading that no serious scholar can accept. Mr. Ivins' thesis is based on his conviction that Vesalius was a knave and that any virtue that the "Fabrica" may possess is to be found in the figures which, on wholly insufficient evidence, he attributes to Jan Calcar. But despite the allegations of Mr. Ivins to the contrary, there would never have been a "Fabrica" had it not been for André Vésale, and modern anatomy dates from the publication of his book. Mr. Ivins is guilty of disgraceful rudeness to several scholars, including Dr. Charles Singer, the eminent medical historian of England, and the late Dr. Harvey Cushing, who perhaps knew more about Vesalius when he died than any other person; he also attacks other scholars, such as Charles D. O'Malley and J. B. de C. M. Saunders. I, too, have been an object of Mr. Ivins' vitriol and I am pleased to find myself in such exceedingly good company. However, it is to be deplored that a scholar of Mr. Ivins' eminence in the field of art should have gone to such pains to abuse every Vesalian scholar, living or dead, when he prefaces his essay by admitting ignorance of anatomy and the Latin tongue.

JOHN F. FULTON

* A volume containing all of the illustrations from the writings of Andreas Vesalius (Tabulae sex, Fabrica, Epitome, etc.) reproduced for the most part from the original wood blocks.

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